

UNCSTD's Year

1979 is the year of UNCSTD, the United Nations Conference on Science and Technology for Development, which will meet in Vienna for two weeks during August. In this past year almost every nation with any interest in science and technology has compiled a national paper representing its point of view, and well over 100 documents, many running to 50 pages or more, now reside in the UNCSTD secretariat at the UN headquarters in New York. During these next few months the secretariat will have the unenviable task of distilling an immense amount of fact and opinion into some sort of agenda for Vienna. In the meantime sub-conferences in preparation for the main meeting continue unabated; in January for instance there will be meetings in Tallin in the Soviet Union and in Singapore (organised by the International Council of Scientific Unions).

Is the conference going to work out satisfactorily? What, even, do we mean by a 'satisfactory' conference? Presumably one that breaks up in fist fights or that falls apart over issues peripheral to the main themes would be deemed unsatisfactory. So too, although this view is not universally shared, would be one in which no voices were raised, no feeling engendered, no criticism voiced. Between these two poles there is much scope and it will depend largely on the leadership that the UNCSTD secretariat gives whether much of value emerges. The outcome of the labours of thousands who have contributed in one way or another to conference documents is, in the strange system in which we work, ultimately dependent on a handful of people at headquarters. In the past year such news that has filtered out has suggested quite strongly that all is not well. If relations are not all they ought to be, then this may well be excessively occupying the time of those who would be better employed in making the conference work.

But what of the papers themselves? After all the very act of compiling national papers must have put some countries through a salutary exercise of asking questions, perhaps for the first time, about the relationship between science, technology and development. We expressed the hope a year ago that this exercise would not be confined to bureaucrats but would encompass as many scientists and technologists as possible, whilst recognising that the conference is not specifically about science and technology as such. It is pleasing to be able

to note that if the preambles to papers are to be believed a widespread process of consultation, meetings and conferences has gone on in many developing countries. Developed countries, cautious about committing themselves to large new expenditures on major institutional change, have on the whole provided papers descriptive of what they can do and are prepared to do but which shy away from more general discussions of principles—some also show signs of having little involved people outside certain official circles. The British paper is a case in point; it is scrupulously accurate but the Overseas Development Ministry, which compiled it has not in any sense used the preparation of the document as an opportunity to get a national debate going.

The Vienna meeting could be an excellent opportunity for the world to evaluate very carefully just what is being and could be done in the field of science, technology and development. But for this to happen some very self-critical voices would need to be raised. Unfortunately those who attend are most likely to be representatives of government agencies and national science funding agencies with a natural concern for self-preservation. Even if the papers contain an element of self-criticism (and a few do, notably from the developing world), when it comes to the meeting itself it will take a very brave delegate to acknowledge publicly—and take the message back home—that national mechanisms for channelling science and technology into development are in need of overhaul. And yet this undoubtedly needs saying. In the developed world there is inadequate discussion about whether the level of scientific resources devoted to the developing world is appropriate and whether present organisations are making the best use of these resources. In the developing world national science bodies too often seem to get into the hands of scientists who steer the country's effort away from the immense complexities of development and towards the slightly less complex business of imitating Western science.

If the UNCSTD secretariat could make delegates ask fundamental questions about their own national efforts, that would be a real achievement. And if the organisers of the peripheral meetings in Vienna could reach out beyond official viewpoints and tap some of the more speculative feeling abroad, then the whole exercise could still be very worthwhile. □



Kenya leads the way in environmental planning

THE government of Kenya has started a systematic across-the-board environmental assessment of its entire development programme—the first national exercise of its kind in the Third World. Officials at the United Nations Environment Programme (UNEP) and the United Nations Development Programme (UNDP), both of which are assisting Kenya in executing the project, are enthusiastic about this project.

Its success should help UNDP to promote similar environmental assessment projects in other developing countries, where many policy makers continue to harbour the same mistrust towards the environmental debate that they displayed at the Stockholm conference in 1972. While many argue that environmental considerations are a luxury for Third World development planners, there are others who see in environmentalism a philosophy designed to retard the economic development of the Third World, particularly its industrial development.

The Kenyans are quite aware that there can be strong conflicts between environment and development. They are therefore looking for only limited but quite realistic results from their exercise. At the least, it should help to reduce those innumerable environmental problems that are often created because of simple oversights at the planning stage.

Kenya's Tana River development scheme, for instance, will create one of the biggest man-made lakes in Africa. The spread of irrigation carries the threat of schistosomiasis. Between disease and agricultural prosperity Kenya, like any other Third World

country, would obviously choose the latter. However, an advance environmental assessment exercise would help to promote possible control measures right from the beginning. It would also remind project authorities that the labour force building the dam has to be provided with energy. Otherwise, merely because of this oversight, forests could get depleted, and the resulting soil erosion could lead to rapid silting of the new reservoir.

Environmentally bad siting of projects, like a chemical plant near a bird sanctuary, can be prevented quite easily with the help of such exercises. "To delay the starting of a paper mill after it has been built, neither helps the country nor the firm setting it up", explains George Muhoho, until recently head of Kenya's National Environment Secretariat (NES), the Kenyan agency co-ordinating the environment-development project.

In the past, the NES has conducted several studies on various individual projects and influenced government decisions. But the current exercise now provides it with a rare opportunity to develop a comprehensive and long-term approach to environmental planning in the country.

The final recommendations of the project may well strongly favour a high rate of industrial growth. The most pressing environmental issues in Kenya are those related to land-use. South of the Sahara, Kenya has the highest population growth rate. Kenya's population is expected to double within 20 years. At the moment, nearly 90% of Kenya's population lives in the rural areas earning its livelihood predominantly from agriculture. Unless a rapid transformation takes place in the occupational structure of the country and more people find jobs in industry, the pressure on the land could become too intense.

As increasingly marginal land is brought under cultivation, deforestation and desertification will increase. Encroachment on game parks could seriously affect wildlife conservation. By contrast, Kenya could simply forget about air pollution control until it has reached a far higher level of industrial

development. A comprehensive environmental planning exercise could thus identify urgent development priorities and critical environmental issues as well.

An important aspect of the project is that it relies largely on local Kenyan expertise. The temptation to invite a UN mission consisting of Western experts to prepare a report on environmental problems was studiously avoided. Such an approach would have robbed the Kenyans of a chance to assess by themselves the environmental effects of what they were doing, and thus develop their own perspective on environment and development related issues. Moreover, even in the West, few people can really stake a claim to be an expert on environment-development planning.

To ensure the involvement of all available local expertise, and popular participation, the project is being executed in three phases. In the first phase, which was completed in August, 1978, five papers were prepared that surveyed major environmental problems in Kenya. These papers were then thrown open for discussions at a week-long meeting in July, 1978, attended by 11 senior Kenyan officials, including the Minister of Finance and Planning, and several permanent secretaries in the government. This meeting identified existing gaps in knowledge and possible policy alternatives. Finally, a steering committee for the rest of the project was formed consisting of the officials present at the meeting to enlist the active co-operation of all the ministries.

In the second phase, much more specialised studies on specific scientific, technical, legal and sociological issues are being conducted to find answers to the questions raised at the earlier meeting. To do these studies, local expertise will be drawn from wherever available—from official institutions to non-government organisations and universities. Foreign experts will be recruited only for highly technical subjects where no local expertise exists. Finally, in the third phase, to be completed by August, 1979, the final recommendations will be put together.

Anil Agrawal



The pressure on land : environmental oversights can limit growth

Bacteriologists lobby GMAG's first public meeting

A LOBBY for the exemption of experiments in which recombinant DNA techniques are used simply to rearrange the genes within *E. coli* and other bacteria made a strong protest against continuing control at GMAG's first public meeting last week.

GMAG, Britain's Genetic Manipulation Advisory Group, was fulfilling its promise to hold open meetings on its proposed new system for the risk assessment of recombinant DNA experiments (*Nature* 276, 104; 1978).

The new chairman of GMAG, Sir William Henderson (above right), opened the meeting optimistically, saying that those who feel that GMAG's hand is too heavy "do not sufficiently allow for the depth of public interest, and how the composition of GMAG takes this into account". But all on GMAG, said Sir William "wish to improve the procedures and consult".

Those at the meeting, held on the last working day before Christmas (22 December), were mostly scientists. The consultation amounted largely to two protests from the floor: that the new scheme was difficult to operate in practice because of lack of sufficient knowledge; and that the re-arrangement of genes within a bacterium such as *E. coli* using recombinant DNA techniques (but not otherwise) must still legally be notified to the Health and Safety Executive and then to GMAG, and must be carried out under Category I conditions.

These conditions, although the least

stringent of GMAG's four categories of physical containment, are still more rigorous than normal laboratory practice and can mean that the laboratory has to be modified. This type of experiment has now been exempted from control under the proposed new NIH guidelines in the US, but hopes that the new proposals in the UK would automatically exempt these experiments have been disappointed.

Question after question from the floor drew an acknowledgment from Professor Mark Richmond of GMAG that the group would have to make a decision on this problem soon. John Maddox, one of GMAG's public interest representatives, defended the categorisation and notification of such experiments on political grounds. While accepting that the procedure for notification would be made simpler and easier, he took the view that public concern would be lessened if GMAG were seen to be controlling all experiments using recombinant DNA techniques, even though there might be scientific grounds for exemptions in certain cases.

From the floor Professor R. Pritchard warned that this type of apparent illogicality undermined the credibility of the whole notification procedure in the eyes of researchers and their local safety committees.

Nevertheless, the feeling of the meeting was that the new risk assessment scheme was more rational than previous guidelines. But its precise application worried people.



Sir William Henderson: optimistic

The most immediate problem is posed by the 'gaps' in biological knowledge which become apparent when a consistent attempt is made to calculate the risk of an experiment. For example, the causes of pathogenicity are not well understood, so one cannot estimate all the possible biological effects of a modified bacterium on the human body.

In cases like this Dr Sydney Brenner—who invented the scheme and was present at the meeting—falls back on averages. Of all known bacteria, what fraction are pathogenic? This fraction could be applied as the probability that any new bacterium were pathogenic.

Dr Peter Rigby of Imperial College, who according to Dr Brenner "has worked out a magnificent set of examples", raised the final issue: that all the calculation is in vain if the precise containment effectiveness of the four containment categories is unknown. Some claim the containment factor between categories I and IV to be 10^{12} ; some 10^8 . "It must be measured" said Dr Rigby, extracting a cautious commitment that GMAG act.

Eleanor Lawrence

UK health ann safety: £1,800 million bill

PREVENTION rather than cure, and hence research, is a major theme in the recently published annual reports of the UK's Health and Safety Commission (HSC) and Health and Safety Executive (HSE). The HSC's plans for the next five years, its report says, include epidemiological studies on populations at risk from hazardous substances; a major review of the risks to health from exposure to asbestos; the development of a policy for protecting people from the hazards of genetic engineering and working with dangerous pathogens; and research on the design safety of equipment in nuclear power reactors.

In the introduction to the HSE report John Locke, the HSE's Director General says that "research, the systematic collection and analysis of a wide range of data and the dissemination of

information to all who need it" is the hallmark of the executive's efforts to reduce occupational hazards. He points out that the 16 million days lost in 1976 through accidents are estimated to have cost the country some £1,800 million. The "great majority of accidents could be prevented if simple precautions are taken" he says.

Over the past few years, the HSC's report says, there has been a reduction in the number of deaths from industrial accidents. Nevertheless, Mr Bill Simpson, Chairman of the commission, believes that "there is no room for complacency". Although the number of deaths has gone down, the number of notifiable accidents was 329,000 in 1977, 2,000 up on the previous year.

The commission's report also describes the preparation of draft regulations on the notification, and in some

cases survey, of installations constituting a major hazard. It comments on the regulations laid before Parliament for the packaging and labelling of about 800 dangerous chemicals in common use and discusses its efforts to change the law which exempts government controlled institutions from prosecution under health and safety legislation.

The problem of the universities and health and safety is still under review by the executive according to John Locke. The HSE is still getting to know people in the universities (heads of departments, trade unionists etc.) and identifying those sections which may present special hazards to workers or the public. Locke feels that students are the main problem because generally they are not sufficiently well trained to work in hazardous environments.

Alastair Hay

Protests over Einstein statue

A PROPOSAL by the US National Academy of Sciences to erect a 21-foot statue of Albert Einstein in its Washington grounds has run into a storm of protest from members of both the academy itself and of the local Washington community.

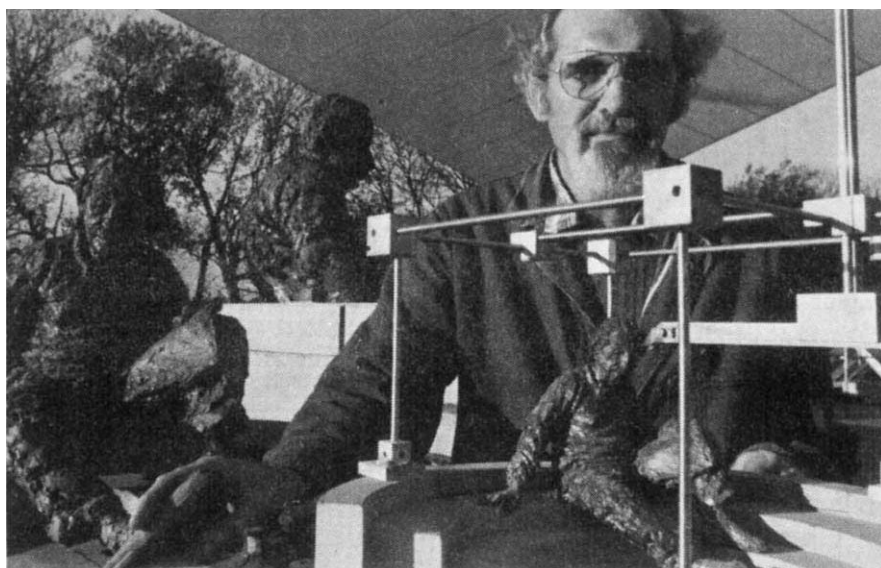
Several physicists have complained that at a time when basic science is being squeezed for funds, it is inappropriate for the academy to be seen spending \$1.5 million on the statue, and that Einstein himself would have preferred the money to have been spent on some scientific endeavour.

Others have pointed out that Einstein repeatedly asked that he should not be treated as a cultural hero. And comments on the design of the statue in the local press have ranged from "a blunder of mediocrity" to "the latest disgrace to our city".

The decision to erect the statue as part of this year's centennial celebrations of Einstein's birth was announced two months ago by Dr Philip Handler, president of the academy. According to Dr Handler, the large size of the bronze statue—which together with its marble base will weigh 139 tons—represents that fact that Einstein was "a giant among men".

The sculpture is the work of artist Robert Berks, who sculpted a head of the physicist in 1953. He had previously proposed unsuccessfully a full-length statue to the Weizmann Institute in Israel, to Princeton University, and to the Smithsonian Institution in Washington.

The academy has written to all its members, as well as other members of



Sculptor Robert Berks with models of the controversial statue

the scientific and technical community, requesting financial support for the project. But while some have been pleased to contribute—over 2,000 contributions have so far been received—others are less happy.

"There are a number of people here who almost hit the ceiling when they were asked to contribute to the statue. So far I have not heard a favourable word from anyone on the scheme," a physicist at one West coast university said last week.

Another complained that the decision to erect the statue had "simply arrived out of the blue", and that although the proposal had initially been put to and accepted by an annual meeting of the academy, there should have been wider discussion among members.

In Washington, opposition to the statue has been expressed mainly in the pages of the *Washington Post*. Fol-

lowing a critical article by one of the newspaper's contributors, a number of readers wrote in voicing objections.

Commenting on the large scale of the statue, for example, one correspondent wrote that "If anything concise may be said about Einstein's life, it is that he was a philosopher of mathematical precision and theoretical proportion". The *Post* itself joined in the fray with an editorial complaining that the statue "doesn't honour its subject", and suggesting that the academy should "start again".

A spokesman for the NAS said last week that the academy had not yet decided whether to reply to the criticism made in the *Post*, and pointed out that a large number of favourable comments about the statue—including its initial approval by Washington's Commission of Fine Arts—had been received.

David Dickson

AAAS to assist China with the popularisation of science

AN agreement opening the way to joint activities on the popularisation of science and on science education has been reached between the American Association for the Advancement of Science and the Scientific and Technical Association (STA) of the People's Republic of China.

The agreement, which is expected to lead to increasing co-operation at the nongovernmental level between the two organisations, was worked out during a visit to China last month by a 12-person delegation from the AAAS.

In particular, the agreement provides for a return visit by the Chinese to the US next spring, and for the AAAS to identify US specialists willing to visit China to lecture on topics specified by the STA.

Announcing these moves in Washing-

ton on the AAAS delegation's return, Mr Emilio Q. Daddario, chairman of the association's board of directors, said that various Chinese ministers had given their approval to such an agreement, and that President Carter's announcement of the normalisation of diplomatic relations between the two countries "cannot but help to make our agreement more meaningful."

Mr Daddario also said that the Chinese were contemplating sending a special mission to the US to look at various ways in which science is "popularised". Such a visit might include talks with science journalists, and visits to science museums and technical information centres.

"During the Cultural Revolution there was a sense in China that science produces an intellectual elite. Now they

want to make it clear to the masses that science has benefits for people, and that it is important that they should understand it", said Mr William Carey, executive officer of the AAAS.

"The Chinese want to get people in the streets, in the farms and in the factories to support the thrust towards modernisation in the fields of science and technology. And they have a very great appetite for US experts to go and give lectures, a method they are seizing upon to get a quick turn-around on their scientific efforts."

Mr Carey said that the Chinese had expressed interest in working with the AAAS on various aspects of science education, in particular the development of curricula for university and higher education.

David Dickson

University of California faces drastic cuts

Proposed budget reductions may force closure of whole campuses and medical schools, writes **John H. Douglas** from San Francisco

RESPONDING to strong popular sentiment against high levels of government spending, California governor Edmund G. Brown, Jr., has demanded that all state agencies submit a list of programmes that can be cut immediately in order to bring about a 10% reduction in next year's budget.

Such cuts could drastically affect the prestige and research capability of the 9-campus University of California, which accepts students from the top 12.5% of the state's high school graduates. More students, however, would be affected by reductions in the 19-campus State University and Colleges system, which accepts students from the top one-third of high school classes, and in the 106 state-supported Community Colleges, which presently are open to any resident 18 years of age or older.

In a strongly worded reply to the governor's demands, University of California president David S. Saxon said flatly that spending reductions of this magnitude simply could not be accomplished by next year if the university were to honour its commitments to present students and to contracts with external agencies. Even to reduce the budget over the next three to five years, he said, would require closing a combination of campuses, medical schools and associated research institutes.

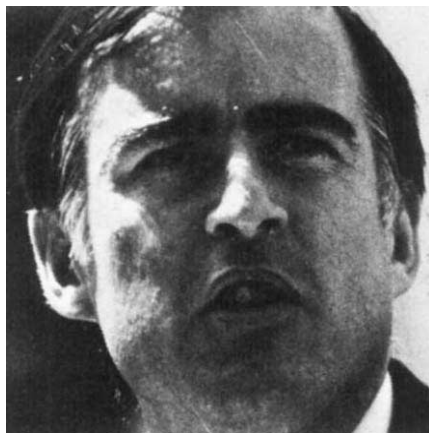
However, one of the factors cited by Governor Brown in defence of his proposed cuts is that during his past four year term the budget for the State University and Colleges has risen by 44.4% over the cost of living, although enrolment has dropped 2.6%. Such declines can be expected to continue, since a 25% reduction in the college-age population is expected over the next decade.

Saxon nevertheless warned the governor that to cut 10% (or about \$77 million) in state support would translate into a much larger reduction in the overall university budget, since many programmes receive an equal amount of funding from other sources. Especially hard hit would be the science programmes that receive large research grants from the Federal government. "The number of jobs lost would be more than doubled", Saxon said.

To make matters even worse, the proposed reductions would come just at a time when the university has already suffered a \$31 million cut in its proposed budget, following passage of Proposition 13 in this year's elections (which substantially reduced property

taxes). And university employees did not receive any salary increases this year, putting them at the mercy of 8% inflation and setting the university a year behind many other institutions. Unless salary increases come soon, says Saxon, "our base would be so low that we would be unable to offer competitive salaries to any employees, and especially faculty, for the foreseeable future".

If major units of the University of California must be closed, speculation has centred on the vulnerability of the two smallest non-specialised campuses, at Riverside and Santa Cruz. The campuses suffered enrolment decreases this year of 5% and 3.5% respectively, compared to an increase in overall university enrolment of 1.5%. Riverside was upgraded from an agricultural institute and has never enjoyed the prestige of older campuses. Santa Cruz is remotely located and uses a controversial student evaluation



Governor Brown: cuts all round

system. Closing either unit would require special legislation, however.

Other solutions under discussion would also require new legislation and, perhaps more important, the co-operation of the governor. Rather than cutting expenses, as directed, the university could be made more independent of state treasury funds by increasing student fees. California now supports the only system of higher education in the United States that is theoretically tuition free, although students pay about \$700 a year in "fees". Increasing such fees by \$100 a quarter (or imposing tuition of that amount) could raise nearly \$35 million for the university. Proponents of this idea point out that more long-term

loans are now available to middle class families than when the tuition-free system was originally set up. Raising admissions standards might also help relieve pressure on the university.

Population decreases and rising fees are likely to hit hardest at the Community Colleges, where competition for entrance is not so keen. Enrolment in normal credit courses declined 4% this year, although it was originally expected to rise 3%. And when new fees were added last fall to non-credit courses (which attract older students not working toward a degree), enrolment in such courses plunged 40%.

All of these problems are likely to come to a head next spring when the California legislature considers the state budget. The eventual outcome is likely to hinge on details of a political process that may seem quaint even by California's arcane standards. Although the universities and colleges have powerful friends in the legislature, the governor has authority to veto specific budget items and could thus eliminate individual programmes he dislikes. And, for the first time in this century, California's governor and lieutenant governor belong to different parties. The new Republican lieutenant governor, Mike Curb, has strongly hinted he will oppose Democrat Brown on the university issue. What this would mean remains unclear.

Finally, several legal questions remain unresolved. By custom, faculty tenure at the University of California has been considered to apply only on one campus. If campuses were eliminated, the future of tenured faculty would be cast into doubt, as would the university's reputation as a desirable institution with which to affiliate. Long-term research contracts between university institutes and Federal funding agencies would also be threatened.

In assessing the possible impact of the proposed budget cuts, some of the most vigorous and articulate support for the University of California has come from the president of a competing private institution: Richard W. Lyman of Stanford University. Calling the University of California system "the greatest publically supported university in the country", Lyman said: "Californians have gone to considerable lengths to try to protect this uniquely fine university from being converted into a mere arm of government. It would be a sad irony indeed if, in the effort to trim government down to defensible size, the university were to be damaged in ways that would require many years of painstaking work to correct, if indeed they could be remedied at all". □

news in brief

NASA abandons attempts to rescue Skylab: The US National Aeronautics and Space Administration announced last week that it was abandoning its efforts to prevent Skylab, the 85-ton space station last used by astronauts in 1974, from falling to earth sometime next autumn.

As a result, the vehicle is expected to break up on entering the earth's atmosphere, scattering between 400 and 500 pieces of metal over a path 4,000 miles long. Some of these will probably strike the earth's surface, but according to the agency "the probability of injury or damage is less than that from meteorites."

Initially planned to remain in orbit until 1983, when it could be reached from a standard space shuttle flight, concern about Skylab's fate arose earlier this year when it was realised that the vehicle was slowing down as a result of the extra drag caused by sun-spot activity, and thus beginning to fall back to earth earlier than anticipated.

NASA had hoped that it could maintain Skylab in orbit until a small booster rocket could be sent up on an early space shuttle mission to take it into a higher orbit. However, problems with Skylab's control equipment, as well as development problems with the space shuttle programme, led to NASA's decision—supported by President Carter—to call off the rescue attempt.

Carter expected to boost defence research and cut back biomedical research: A major increase in financial support for military research and development, and a reduction in funding for biomedical research, are expected to be included in the budget request for the financial year 1980 which President Carter will present to Congress on 22 January.

The research and development budget is therefore expected to reflect the main thrusts of the overall budget, with reductions in social welfare programmes in an attempt to keep the budget deficit below \$30 billion, but an increase in defence spending.

It is thought that total funding for military R & D will increase from \$12.6 billion in the current financial year to over \$13.5 billion. Within this figure, about \$3 billion will be allocated to basic research, an increase of 15% over this year, and 7% above the inflation rate.

One administration official is quoted in *Business Week* as saying that "one of the very few certainties about the new budget right now is that basic research and exploratory development money will be downright abundant."

As far as biomedical research is concerned, the Office of Management and Budget is reported to have recommended that the budget of the National Institutes of Health be reduced from its current level of \$3.25 billion to \$3.03 billion. NIH had asked for \$3.22 billion.

Memorial to Birmingham smallpox professor: Birmingham University is to set up a memorial fund to the late Professor Henry Bedson, who committed suicide earlier this year after Janet Parker, a medical photographer, died of smallpox virus which probably came from Bedson's laboratory. Professor Owen Lyndon Wade, Dean of the Faculty of Medicine and Dentistry at Birmingham, proposed the fund, and he hopes that it will provide money for junior staff at the university to travel abroad and undertake research, especially in virology.

In a statement, Professor Wade says that the scholarships would reflect Henry Bedson's care for the underprivileged in the third world. "Henry Bedson was a deeply conscientious man, constantly aware of the humane aspects of his work. His service to the World Health Organisation in the smallpox eradication campaign was outstanding,

both in the laboratory and in remote areas of Pakistan and Afghanistan," he said. All who wish to give support to the fund are asked to send their contributions to the Dean's office at Birmingham University Medical School.

'Refusnik' refused: Viktor Brailovskii (right), who in 1977 succeeded Mark Azbel' as organiser of the Sunday seminar for Jewish refusnik scientists, has now been told by the tax authorities that he can no longer give private lessons in mathematics—his sole source of income since he was dismissed from a senior post as a computer scientist in 1972. The tax department said that Brailovskii could not give such lessons without a teacher's licence. No licence was issued, and Brailovskii could therefore at any time face a prison sentence for "parasitism"—i.e., being without visible approved means of support.

Meanwhile, his mathematician wife Irina (right), has received complete security clearance from the Rector of Moscow University, Anatolii Luganov. However, although the chief emigration office of the USSR has accepted the fact of her clearance, she was informed that this made no difference and she would still not be permitted to emigrate.



West Germany to build fast breeder reactor: Chancellor Helmut Schmidt's plan to build a fast breeder nuclear reactor at Kalkar, near the Dutch border, has been approved by the Bundestag, West Germany's lower House of Parliament. The plan was approved in a close vote of 230 to 225, but only after six members of the Free Democratic Party, which is in a coalition with the Social Democrats, finally agreed to abstain, after threatening to vote against the project.

The vote was crucial to Chancellor Schmidt because he believes that nuclear power is vital to West Germany's economic future. However, he faces strong opposition among the members of his Social Democratic Party, especially since last year when the Party chose coal as the chief source of energy in the future and called for a six-year moratorium on building nuclear plants.

Upjohn scientists induce bacteria to produce chick albumin: Scientists at the Upjohn Co., the US-based pharmaceutical manufacturer, claim to have used recombinant DNA techniques to induce bacteria to produce chicken albumin, a protein considerably larger and more complex than previous molecules produced in the same way.

Reporting their results in the Proceedings of the *National Academy of Sciences*, the scientists say that by transplanting a particular gene into bacteria, about 1.5 per cent of the bacteria's protein production becomes chicken albumin, and they expect that this level can be increased to 10 per cent.

According to the company, the experiment lays the groundwork for producing medically-useful quantities of enzymes and hormones, such as the use of bacteria to make the human blood-clotting factor missing from the blood of haemophiliacs.

A refuge for scientists' writings

Professor Margaret Gowing, Honorary Director of the Contemporary Scientific Archives Centre, Oxford, explains how the centre was set up and outlines its work

History is essential to society and science and technology are crucial ingredients of history. History depends on records and therefore scientific and technological records should be systematically preserved. Until recently however records of 20th century scientists and engineers attracted much less interest than those of, say, politicians or novelists.

Most scientists and engineers work in institutions—government establishments, industrial firms or universities—and if all these institutions systematically gathered and retained records the residual problem would be small. But many of them do not. Thus, few British universities have archives staff and fewer still gather the papers of academics as they retire or die.

For these reasons, the Royal Society established a Committee on Scientific and Technological Records in 1967, under the chairmanship first of Sir Harold Hartley and then of Professor Nicholas Kurti. In 1969 a conference called by the committee agreed that scientists' and engineers' papers required special action, partly because general archives tended to be frightened by them. It was further agreed to establish not a special archive but a centre which would locate, sort and catalogue the papers of individual eminent scientists and engineers. Each collection would then be permanently deposited in an appropriate existing library and only the catalogues, reproduced by the Royal Commission on Historical Manuscripts, would be kept centrally and in the copyright libraries. The Wolfson Foundation gave a grant for an experimental period and I was asked to establish a centre at Oxford, where I had just been appointed Professor of the History of Science.

The Contemporary Scientific Archives Centre started in one basement room in April 1973 and has had a total paid staff of two people who do everything from typing, stamp-licking and driving to collect papers, to producing scholarly catalogues and orderly boxed collections, often from extensive and chaotic manuscripts. By the end of August 1978 60 collections had been handed over to 29 different libraries and a further 14 collections were in hand. In addition 175 enquiries had been made. The list at the end shows the variety of disciplines covered.

The collections cover scientists (including medical scientists) and engineers who died after the Second World

War. The danger point for dispersal of papers is usually retirement but because of shortage of resources the centre can only deal with a very few collections of people as they retire. The Royal Society Committee decides who shall be included but the list is not confined to Fellows of the Royal Society nor do all Fellows automatically qualify.

By 'papers' the centre does not mean only scientific publications. The word includes papers of all kinds such as notebooks, notes of lectures heard or delivered, papers and correspondence covering all stages and all aspects of a scientist's life from school reports to political activity. The collections vary greatly in size and context. Some scientists keep few papers and some papers regrettably disappear: for example, the contents of the filing cabinets of Nobel prizewinner Cecil Powell, the physicist, were shredded soon after his death. By contrast some collections are very large, cover many years and are of outstanding interest: for example the papers of physicist Rudolf Peierls (including a life-long correspondence with Neils Bohr and Hans Bethe); Charles Coulson (including, besides papers on chemistry and mathematics, his many concerns such as pacifism, Oxfam and Christianity) or Patrick Blackett, the physicist (including his scientific, wartime, Labour Party and overseas activities). Some collections are beginning to form a

'critical mass' for the history of a discipline—for example in computing science.

The formula for the centre has proved very cost-effective, while Oxford University has helped greatly with administrative services and accommodation. Location in Oxford has also brought unstinting help and advice from many individuals. Nevertheless finance has been a constant anxiety, for the initial grants from the Wolfson Foundation and other funds were pump primers. At present the Royal Society and the British Library share the main cost with a smaller grant from the Council of Engineering Institutions. For two years from 1979 the Wellcome Trust will replace the British Library grant.

The centre has blazed a trail. Neither of the two staff members had any previous archival or scientific training but their work has been outstanding in quality and quantity and has attracted considerable interest abroad, especially in North America. The Wellcome Trust is now itself establishing a project on similar lines for medical practitioners and medical-related scientists. The work is becoming more valuable as more professional groups—such as various sections of physicists, molecular biologists, and computer scientists—become increasingly interested in the recent history of their disciplines and as more historians study not only the purely scientific work but the other public work and private interests of scientists and engineers. □

For further information and copies of Progress Reports (April and October annually) please write to Mrs Alton, Deputy Director, CSAC, 10 Keble Road, Oxford.

Collections completed and *in hand

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Environmental research in Northern Finland

Peter D. Moore, plant scientist at King's College, London, reports from Finland.

JUST 150 km south of the Arctic Circle, Oulu in Finland is one of the most northerly university cities in the world. It lies on the Bothnian Bay, the northernmost spur of the Baltic Sea, and it has provided a centre for a variety of environmental research programmes in recent years.

The Bothnian Bay is covered by ice for between 150 to 200 days of the year, often to a depth of 4 m and has a salinity in its epilimnion of less than 0.2%. It is an unusual aquatic system. Oulu is an industrial city with a population of almost 94,000, so sewage and industrial effluent is a matter for concern. Research conducted by the University of Oulu at the Krunnit and Perämeri Research Stations on islands in the Bothnian Bay, have revealed that levels of inorganic nitrogen and phosphorus in the waters around Oulu are twice as high ($200\text{--}250\text{ }\mu\text{g l}^{-1}$) as those 40 km north. The annual primary productivity is about 32 gm^{-2} in the eutrophicated waters; this is twice as great as that found in the purer waters, but it is nevertheless only about a third of that found in the southern Baltic.

The low annual productivity can in part be accounted for by the prolonged frozen period, but even when liquid, the discolouring effect of humus colloids in the waters of the Bothnian Bay result in poor light penetration. The maximum depth for 1% penetration of light in summer is only 8–9 m.

The islands in the Bothnian Bay are rich in plant and animal life. On the island of Hailuoto the elk reaches its highest population density in Finland, about one elk per square km. Lichens and macro-fungi on the island have been extensively exploited. Of the lichens, *Cladonia alpestris* is valuable in horticulture and since it forms dense carpets beneath the pine forests which cover the dunes of Hailuoto, harvesting



by hand is relatively simple. Up to 20% of the cover is removed. It is then left for five years to regrow, the growth rate being about $4\text{--}6\text{ mm yr}^{-1}$. About 800 metric tons are harvested in Finland annually. The island of Hailuoto is also one of the richest sources of fungi in northern Finland. Estimates place the annual fresh weight yield at 200 kg ha^{-1} . Yields further north and east in Finland fall off to around $50\text{--}60\text{ kg ha}^{-1}$, but in the south west they can reach as high as 260 kg ha^{-1} .

Oulu University has a field station on the west coast of Hailuoto from which survey work is conducted and it has a second research station in the east of Finland in the Oulanka National Park, just outside the Arctic Circle. Here the dense spruce forests with thick layers of moss were used as a site for intensive study in the International Biological Programme by Dr Paavo Havas.

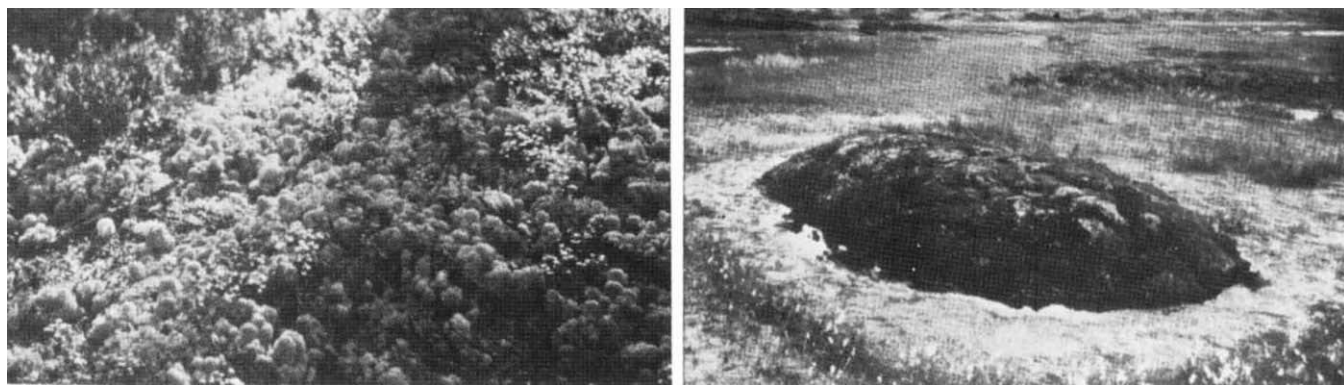
The area is of particular interest to botanists because of the presence of calcium containing metamorphic rocks which permit the development of calcicolous plants such as *Cypripedium calceolatum* and *Kobresia simpli-*

ciuscula. It may be the abundance of cations in local mires which has led to the survival in one inland spot of a population of *Triglochin maritima*, which is normally a coastal species. The existence of such a maritime plant in the Oulanka park led plant geographers at Oulu to speculate about the existence of a sea link between the Baltic and the White Sea when the area was depressed by glacial ice. Dr A. Vasari has been able to trace macroscopic subfossil remains of *Triglochin maritima* in the peats of its current locality going back 8,000 years. On the other hand, there are a number of maritime species, for example *Armeria maritima* and *Plantago maritima*, which have inland (often montane) ecotypes which were widespread during the closing stages of glaciation. *Triglochin maritima* could be another species of that type.

Finnish Lapland can boast yet another research station, this time belonging to the University of Turku, at Kevo in the far north of the country. It lies some 60 km north of the continuous pine forest in sub-arctic birch woods and fjell tundra. In this area, approaching 70°N , permanent ice cores are found within mounds, termed palsas, which typify the peat-land habitats.

Much speculation has surrounded these palsa mires, in particular the circumstances under which they were created. Some people, such as M. Salmi (Turku) believe that all palsas began their formation at specific times in the past. He claims that the Lapland palsas fall into two groups which were initiated about 5,000 and 3,000 years ago respectively. Palsa studies in Ontario, Canada, by J. B. RAILTON and J. H. SPARLING (Toronto), however, indicate a continuous and non-phased cycle of palsa initiation, growth and erosion.

M. Seppälä, working at the Kevo Subarctic Research Institute has introduced a novel, experimental technique into the discussion. His observations led him to believe that palsa mounds



Left: a patch of *Cladonia alpestris*; right: growing palsa hummock

are created as a result of a failure of snow to accumulate on certain parts of the bog surface, so allowing ice penetration of those parts to be deeper. In winter, the wind blows the snow unevenly over the mire and, when temperatures fall to less than -40°C , frost may penetrate to depths of 1 m.

If this explanation is correct, then it should be possible to create palsas experimentally by regular snow removal. Seppälä has contrived such an experiment and has kept experimental

plots clear of snow during the past two winters. In the first winter, frost penetrated to 85 cm in the cleared area and only to 45 cm in the control. By mid August the control was ice free but the experimental plot was still frozen to a depth of 45 cm. In the second winter the artificial palsa had an ice core 100 cm deep and ice was still surviving to about 60 cm this September. The expansion of the ice core has now led to the surface of the mire in the cleared area being elevated

20 cm above its surroundings. This in turn has resulted in a natural wind clearance of the winter snow leading to more rapid palsa growth.

Studies such as these, together with lichen productivity, reindeer ecology and arctic nutrient cycling processes, all being carried out in Lapland's research stations, will undoubtedly contribute much to our lamentably poor understanding of the ecosystems of the far north. □

Down in a Polish forest, something is stirring...

SOUTHERN Poland, from an ecologist's point of view, could well be a nightmare—a jumble of conurbations, industrial enterprises and national parks, stretching in a wide belt across the country, while the high Sudeten-Carpathian mountain barrier effectively inhibits north-south ventilation. Not surprisingly, the forests of the area have deteriorated, and research into their management has been ranked as a "key" project.

In 1976, a detailed survey was begun into the management of these forests, with the participation of thirteen research institutions—four from the Polish Academy of Sciences, seven from the Jagellonian University and other academic institutions in Krakow, and two non-academic institutions (the Institute of Meteorology and Water Management and the Institute of Environmental Protection.) Some 70 senior research workers are involved in the project, ranging from ecologists and dendrologists to geochemists and economists.

The area chosen for study was the Niepolomice forest, some 30 km east of Krakow. The forest consists of two sections, a northern, deciduous part (~ 3,000 ha) and a southern coniferous part (~ 8,000 ha) mostly pine. Conifers are the most susceptible to pollution.

The forest, formerly a royal hunting park, is of considerable historical and touristic significance. It is ecologically unfortunate, therefore, that in the late 1940s, the new industrial town of Nowa Huta, with a steel mill still the largest in Poland, was established between Krakow and the Niepolomice forest. The prevailing wind is from the west, so that the forest receives the major proportion of industrial emission from Nowa Huta.

The siting of Nowa Huta was apparently primarily a politico-demographic decision, at a time when large-scale enterprises were seen to be the answer to the problem of rebuilding a war-shattered economy, at a time when

no government anywhere was more than dimly aware of the concept of ecology as a planning factor.

After 25 years, however, the Niepolomice Forest was unhealthy, and a five-year project was undertaken, not only to find a remedy for Niepolomice, but also to establish a detailed mathematical model of the forest eco-system, and to propose methods of forest management in urban environments which could be extended to similar systems elsewhere.

On the basis of this computer model, a number of parameters were selected for field study, including dust-fall, wet and dry deposition of sulphur, heavy metal contamination, acidification of tree bark, loss of photosynthetic efficiency of pine-needles, energy flow via small consumers and predators, and the quality of roe-deer antlers. The situation was found to be far from promising: dust fall 45–80 tonnes/km²-yr, wet and dry sulphur deposition 3.6–6.4 tonnes/km²-yr, potential photosynthetic activity of pine-needles decreased by 13–18%. The total energy flow diagram revealed a slowing down of decomposition, producing a "carpet effect" under the trees, since the pine litter takes up to 10 years to break down completely, instead of the usual two, apparently due to inhibition of microbial activity, possibly due to heavy metal pollution.

Recent introduction of electrostatic precipitators in the Nowa Huta factories is improving the dust situation to a certain extent, but the problem of wet and dry sulphur deposition remains, so far, virtually insoluble. One proposal made by the research team is aerial spraying of the forest with lime, neutralising the sulphurous and sulphuric acid. This is being introduced into the nutrient spraying programme which has been carried out over the last three years and which is already proving a palliative—the timber increment has increased somewhat and the overall condition of the pines is im-

proving. (Unfortunately, there is also the side effect of increased weed-growth and the eutrophication of the small river Drivnka).

In addition to this spraying, the team have put forward several other draft proposals. The forest stands, they say, should be rebuilt into a mixed forest with the possible introduction of exotic species such as black pine and Japanese larch at the western edge. (It is hoped in the course of the next ten years to determine the most resistant strains). The water-table should be raised by regulating the drainage channels in order to provide more available nutrients for the roots. Wild-life management (roe deer, red deer, wild boar), should be optimised, and steps should be taken to reduce tourist pressure by organising 'nature trails' on the British model.

A number of major problems still, of course, remain. For example, the net sulphur intake of the total biomass is still ~2 kg/year and the well-being of the forest can only finally be ensured by the radical control of industrial emissions. Nevertheless, the valuable contributions of the multidisciplinary team towards containing this problem is already effecting some amelioration, and at the same time has produced a valuable spin-off in the form of a number of published papers, and a book now in production, dealing with basic concepts of forest ecology and management.

Vera Rich



correspondence

Pitldown hoax: new light

SIR,—If L. B. Halstead (2 November, page 11) had taken the trouble to look a little more closely at the evidence presented in the scientific papers on the Pitldown forgery (as well as in my book, *The Pitldown Hoax*) he would surely have realised (and this goes also for Mr Richard L. E. Ford) that the late Professor Douglas' arraignment of Professor Sollas is really without any foundation. The "evidence" of Sollas as instigator and co-conspirator with Charles Dawson amounts to very little and that, quite trivial and unconvincing.

Dr Oakley has already, in his letter to *The Times* (7 November 1978) shown that Douglas was mistaken in thinking that the Mastodon bones he said he gave Sollas were part of the Pitldown assemblage. The receipt by Sollas, according to Douglas' recollection of 68 years ago of a packet of potassium bichromate can hardly be taken as positive evidence for the reasons which Dr Oakley gives in his letter.

Moreover, Douglas should have known that Dawson had admitted to the use of bichromate, that Woodward knew this, and two people told me spontaneously (that is before the disclosure of the hoax) that they were aware of Dawson's staining activities. Moreover, as Oakley and I have shown, Dawson used a number of different methods of staining and we are left to suppose (on no evidence whatever) that Sollas initiated Dawson in all these methods.

The third item furnished by Douglas in his remembrance of Sollas borrowing apes teeth from the Anatomy Department at Oxford. If Douglas had remembered Sollas borrowing an ape's jaw or even better, an orang's jaw, that would have been rather more interesting, because it was an orang jaw with its molars that was used for the plants at the two Pitldown sites. To say that Dawson needed Sollas as a supplier is sheer guess work and unnecessary at that.

A likely source, as I have pointed out, was Abbott of Hastings, not to mention local museums and even straightforward purchase. A fourth item of "evidence" is the Sherbourne horse's head. For Douglas to say that this little curiosity (which cannot in fact be brought home to Sollas) is "an almost similar act" to that of the elaborate Pitldown Forgery is quite fatuous.

The motive attributed to Sollas for the humiliation of Smith-Woodward does not make sense. If his plan was to "expose" Smith-Woodward's gullibility and incompetence, why should he have so firmly supported the new *Eoanthropus dawsoni* in the first place? Why did he not wreak his vengeance by exposing the forgery at any time after Dawson's death in 1916? Halstead argues disingenuously that once Dart had discovered *Australopithecus* Sollas would have known that it was only a matter of time before Pitldown Man was exposed. But if Sollas was so sure about the status of *Australopithecus africanus* in relation to

Pitldown, surely Dart's discovery provided the perfect opportunity (from 1925 onwards) to expose both Pitldown and Smith-Woodward.

The truth is that none of the leading palaeontologists of the day, including Dart and Broom (despite their many australopithecine discoveries), nor Leakey, nor Le Gros Clark, could see that *Australopithecus* made Pitldown Man impossible and (*ergo*) fraudulent. The reasons for this myopia, I have discussed elsewhere. A hint from Sollas or later Douglas, to any one of these would have been sufficient. Halstead is simply arguing from hindsight in suggesting that Sollas went to his grave rubbing his hands in anticipatory glee.

In my book I point out in some detail that a "mastermind" to guide Dawson through all the years of the complicated sequence of the events at Barkham Manor and Sheffield Park would need to be of Mephistophelean calibre. It would be tedious to repeat here all that is implied in asserting, or postulating, a continuous connection between Dawson and Sollas. Douglas and his sponsors do not even begin to face up to this. In the case of Sollas it would be quite as difficult a task as that which faced the recent accuser of Elliott Smith. Messrs. Halstead and Ford might care to look for proof of Sollas' alleged activities in the light of the arguments in my paper on Elliott Smith and Pitldown (*Symp. Zool. Soc. Lond.* no. 33, 23–26; 1973).

One thing the late Professor Douglas has succeeded in doing. He has certainly added a mystery of his own devising to the Pitldown saga—why should Douglas on such incredibly weak evidence take the trouble to besmirch Sollas' reputation?

Yours faithfully,

J. S. WEINER

*The Athenaeum,
Pall Mall,
London*

Proliferation

SIR,—In your article 'Is Proliferation Unstoppable' (26 October, page 681), you say that after the May 1975 Review Conference of the Treaty on the Non-Proliferation of Nuclear Weapons "... no outsider proceeded to sign". You cite this as evidence that "the NPT could hardly be said to be an unqualified success".

The facts are that during and after the conference eleven additional states joined the NPT. The new states included Libya, Venezuela, Japan, Switzerland and Portugal. A growth of 14%, including some important states, is not bad going. The treaty now has 104 members and Indonesia and Turkey are in the process of ratification.

Only 13 non-nuclear weapon states that have any significant nuclear plant remain outside the treaty; they include several of those mentioned in your article. However, in eight of these 13 every nuclear plant of which we are aware is under safeguards pursuant to

non-NPT arrangements (like the German/Brazilian agreement). Only five non-nuclear weapon states have unsafeguarded plant—these include India, Israel and South Africa.

We agree that everything should be done to strengthen the non-proliferation regime, but one should not denigrate the considerable success of the treaty. A few years ago, German and Japanese ratification was by no means certain; now almost every leading industrial non-nuclear weapon state is a party. Moreover, IAEA safeguards have become much more effective and stringent in recent years.

Yours faithfully,

HANS-FRIEDRICH MEYER

IAEA, Vienna, Austria.

Responsible statements

SIR,—Though *Nature* is to be applauded for welcoming presentation of alternative views on controversial issues, I would think that the nature of the journal requires the editors to hold authors to a reasonable standard of responsibility, even on issues that evoke ideological conflicts. I therefore wonder why you lend the dignity of your pages to such statements as the following, in an article by Jonathan King (2 November, page 7): "Now we don't have a law in the United States that keeps anybody from introducing any gene into an epidemic strain of *E. coli* or *salmonella* or *Shigella*; and there are forces in that direction because of patents. If somebody else has a patent on making insulin in *E. coli* and you recognise that market what do you do?"

Yours faithfully,

BERNARD D. DAVIS

*Harvard Medical School,
Boston, Ma., USA*

Helpful oil companies

SIR,—The article on wave energy (30 November, page 433), gives a poor impression of the helpfulness of the oil companies, which I would like to correct.

UK OOA wave data is commercial and confidential, and there was a time when Wavepower Limited could not have access to it. However, that access was cleared some time ago via the Department of Energy—I was misinformed, and misled your reporter.

Nevertheless you misrepresent both the oil companies and myself when you say that I have been unable even to arrange a visit to one of the platforms. My enquiry at the conference was the first time of asking, and I have already had two offers in reply.

Yours faithfully,

M. J. PLATTS

*Wavepower Limited,
Southampton, UK.*

news and views

Spacecraft charging

from Gordon L. Wrenn

HALLOWEEN is traditionally the time when things go bump in the night and was somehow appropriate for a Spacecraft Charging Technology Conference sponsored jointly by the United States Air Force and NASA. Justification for the three day gathering of some 150 scientists and engineers really stems, not from physical bumps but from a host of status anomalies seen on operational spacecraft at high altitudes. The proposition is that these malfunctions, ranging from nuisance level to a possible fatality, are caused by electrostatic charging events which occur near local midnight when a satellite passes through the shadow of the Earth. Six sessions at the impressive Air Force Academy in Colorado Springs served up 54 well presented papers; these demonstrated the obvious interest and support that the field has attracted. Why, then did the Conference close with a panel discussion on the theme 'The Spacecraft Charging Hazard—is there a credibility gap?'; furthermore why was the only answer 'Yes'.

The electrostatic potential of any satellite will float to a value which produces zero net current. At low altitudes, a high concentration of cold ionospheric plasma clamps the floating potential at a small negative value. Up at several Earth radii, outside the plasmasphere, the cold plasma decreases, the photoemission current due to sunlight becomes dominant and the spacecraft charges to a few volts positive. In eclipse negative charging is to be expected but the absence of cold protons could permit a potential build up to very high values—20 kV has been observed. The geosynchronous orbit (at 6.6 R_E) is a popular one for a variety of applications satellites but with eclipse times of up to 75 minutes and a location at which intense fluxes of high energy electrons are encountered

during periods of geomagnetic disturbance, it seems to be particularly susceptible to charging. The problem is complicated by the multiplicity of surface materials in use. In the same way that there is little danger when lightning strikes an aircraft, overall charging is not a threat but the possibility of differential charging presents the real hazard; subsequent discharge could generate a large current spike on power or ground lines and affect sensitive solid state devices.

Since the last such conference in October 1976, much good work has been done on modelling both the particle environment and electrostatic fields around spacecraft. Considerable advances have been made in understanding surface material properties and developing active potential control systems utilising electron or ion beam emitters. Studies are now well in hand for huge space platforms such as solar power satellites with areas over 100 km², and charging characteristics will clearly be of utmost importance to their design. One outcome of the earlier conference was a resolution to include simple discharge monitors on all operational spacecraft but this has been largely ignored and the study of anomalies has progressed little. Satellite designers have not been convinced that the hazard is serious enough to justify increased production costs.

The scepticism is bred by a number of missing links in the whole discussion. A considerable catalogue of anomalies does have some correlation with geomagnetic activity but generally does not show an expected pattern with local time. Evidence for kilovolt charging comes exclusively from the ATS 5 and ATS 6 satellites but even these exhibited no corresponding status anomalies. There have been many other suitably instrumented satellites for which charging events have remained absent or undetected. Laboratory simulation of the charging process is difficult

and has not yet been achieved on a system level. NASA and USAF hope to obtain all the answers with the SCATHA satellite which is due to be launched in January 1979; it promises a concerted attack on all the factors which make up the charging equation. Whilst it necessarily represents a compromise between the desire to create charging problems for study and the need to pursue techniques which might overcome these same problems, it should mark a big step forward on the trail of missing links.

ESA, the European Space Agency, is in a strong position to contribute to the subject. In addition to having a good background in the physics of space materials, it has, in orbit, OTS and METEOSAT spacecraft with the opportunity for careful monitoring of anomalies and it has GEOS 2. In contrast to ATS, the electromagnetic cleanliness of GEOS is excellent. Over 96% of the surface has been made conducting by the use of special paints and an indium oxide coating for the solar array. Differential charging has been avoided but satisfactory thermal characteristics have been achieved and for the first time reliable cold plasma measurements are available at 6.6 R_E . So far no significant charging events (in excess of about 150 V) have been seen on GEOS and it may be that its overall susceptibility is very different from that of ATS. If this is true, it is probably due to different secondary emission properties of the resulting retarded impact energy, the secondary electron yield exceeds one. Selection of suitable secondary emitting surfaces could provide an inexpensive method of limiting the extent of charging.

It is planned that GEOS and SCATHA experimenters should collaborate closely on data interpretation and this presents the best chance of closing any credibility gaps. As to the real significance of the whole issue, it

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could be that the approaching solar maximum will provide the opportunity for everyone to judge; our lives are

now much more dependent upon synchronous satellites than they were 11 years ago. □

Membranes and parasites

from D. F. H. Wallach

A WORKSHOP on the membrane pathobiology of tropical diseases held recently* brought together experts in the various diseases and membrane biologists, biophysicists, biochemists and immunologists to discuss ideas and techniques concerning membrane structure and physiology in relation to problems of parasite/host interactions.

It became evident very early in the meeting that membrane biology represents a major area of common ground for all the major parasitic diseases. Membrane studies are relevant to the modes of action of antiparasitic drugs, and membrane-bound antigens may be of particular importance in the development of serodiagnosis and immunoprophylaxis.

Intracellular parasites, such as the plasmodia which cause malaria, interact with the host cell membrane during entry and exit from the cell, and may modify the membrane during their intracellular development. These different processes may be subject to interference by immunological or chemotherapeutic methods. At least three organisms of those under consideration, *Mycobacterium leprae*, *Leishmania* and *S. American* trypanosomes, are taken up primarily by macrophages by phagocytosis, but have evolved mechanisms by which they evade the normal destruction of foreign material within phagolysosomes. These organisms may also avoid immunological defence mechanisms through their sequestered intracellular location.

The presentations on extracellular parasites revealed additional mechanisms which help them to evade host immune defences. African trypanosomes have the capacity for extensive variation of the chemical structure and antigenic characteristics of a glycoprotein surface coat lying outside the plasma membrane, and invading schistosomes have the remarkable ability to incorporate into their surface membranes a variety of host plasma membrane components, including blood group substances and components of major histocompatibility antigens, mimicking host surface membrane anti-

genicity, and to shed, at least *in vitro*, surface membrane material in a manner reminiscent of the behaviour of some tumour cells.

The workshop revealed that, although extensive immunological information exists on changes in the plasma membranes of host cells and the surface coats or membranes of pathogens during the processes of infection, evidence on the biochemical, biophysical, physiological, genetical and micromorphological processes involved is meagre or totally absent. However, recent developments now permit these approaches to studies of parasite infection. For example a major causative agent of human malaria, *Pl. falciparum*, can now be cultivated continuously *in vitro*, the technique has been established in several laboratories around the world and its expansion to mass-cultivation seems feasible. The major steps in the fractionation of host cell (erythrocyte) and parasite antigens in a model monkey malaria have been achieved; parasite modification of host cell membranes can be analysed both biochemically and immunochemically and the system allows rigorous biochemical analysis of parasite, parasite-induced and host-cell-modified membrane lipids and proteins, as well as numerous other metabolic variables. Although it has not yet been possible to cultivate *M. leprae in vitro*, the organism has been found to grow well in the liver and spleen of most forms of nine-banded armadillos. This permits production of large amounts of organisms free from host cell material, and studies are in progress which should eventually lead to vaccination and improved chemotherapy. Studies of the amastigote forms of leishmania growing within macrophages have revealed that some forms of leishmania modify the surface of host cells in a fashion which is recognisable immunologically. Some forms of trypanosomes can be cultivated either in the absence of cells, or using host cells only as 'feeder' cells. Some forms of *S. American* trypanosomes can now be cultivated on a scale adequate to permit isolation of mutants, setting the stage for genetic experimentation. Moreover, fractionation and biochemical studies, involving both surface and cytoplasmic membranes, have been initiated using these organisms,

as well as leishmania.

Animal models exist for schistosomiasis and, although some 'conventional' biochemical/molecular biological approaches appear less readily applicable to this area, the discussions pointed to the existence of an important group of microtechniques, including micropuncture, microcatheterisation and X-ray microanalysis, which are immediately available to the resolution of critical areas in schistosomiasis. Many of these approaches appear equally relevant to filariasis.

The workshop showed that, in spite of promising beginnings in basic research on the six major diseases, there are important lacunae in information about both parasite and host-cell membranes, which might be filled by application of techniques for membrane studies which are now standard in many laboratories. Present knowledge on the relationships of intracellular parasites within the host cell raise many pertinent questions regarding transport mechanisms within the parasite/host cell complex, particularly of K^+ , Na^+ and Ca^{2+} transport and the ionic preferences of intracellular compartments. These issues could be resolved by flux analyses, possibly combined with specific ionophores, and also by use of intracellular ion-localisation techniques. The effects of possible parasite-induced alterations of Ca^{2+} -fluxes deserve major attention. In another vein, the major anion-transport protein of erythrocyte membranes has been extensively studied, many of its active sites can be specifically labelled and application of these approaches to the membranes of erythrocytes parasitised by malarial parasites is practicable. Such labelling methods could also fruitfully be extended to macrophages (normal and infected) and to schistosomes.

Most participants felt that further progress in the understanding of the six diseases required basic research in the following areas.

Host and parasite surface receptors; parasite surface coats; passage of intracellular parasites into and out of cells; fractionation of host-cell and parasite membranes; membrane composition of host cell and parasite; antigenic variation in parasite and parasite-induced antigens, and immune mechanisms; application of the hybridoma technique to the study of parasite antigens; transport and permeability; membrane enzymes; genetics; propagation of the pathogenic organisms involved; model systems.

Scientists interested in collaborating

D. F. H. Wallach is Director of the Radiobiology Division, Tufts University School of Medicine, New England Medical Center Hospital, Boston.

*A workshop entitled The Membrane Pathobiology of Tropical Diseases, sponsored by the Special Programme for Research and Training in Tropical Diseases of the World Health Organisation was held at Titisee, Federal Republic of Germany on 4-8 October, 1978.

in these or other activities of the Special Programme should contact the Director, Special Programme for Research and Training in Tropical Diseases, World Health Organisation, Geneva, Switzerland, for further information.

Virus precursor shedding from mammary tumour cells

from A. J. McClelland

IN 1976 Ritzi *et al.* made the intriguing observation that mice with murine mammary tumour virus-induced mammary tumours had much greater amounts of the major mammary tumour virus (MuMTV) structural glycoprotein gp52 in their blood than did mice with no mammary tumours. This was true even when the tumour-free mice were productively infected with MuMTV and had very high levels of gp52 in their milk. Ritzi's work raised several interesting questions. How are the MuMTV glycoproteins synthesised and assembled, what is the mechanism and biological function of gp52 secretion by mammary tumour cells and are there differences in the synthesis and assembly of MuMTV in normal and transformed cells?

Thanks to the establishment of continuous MuMTV-producing mammary tumour cell lines (Ringold *et al. Virology* **65**, 135; 1975; Sarkar *et al. Virology* **77**, 12; 1977) some answers to the first question at least have been found. It appears that the MuMTV glycoproteins are derived from a single precursor molecule, designated pre-gp70 (molecular weight 70,000) which is then cleaved to form the glycoproteins used in virus assembly (Racevskis & Sarkar *J. Virol.* **25**, 374; 1978; Schochetman *et al. Virology* **85**, 168; 1978; Dickson & Atterwill *J. Virol.* **26**, 660; 1978). Even more interesting is the unexpected discovery that the MuMTV glycoprotein precursor is shed from the surface membrane of the tumour cells in which it is synthesised.

Racevskis and Sarkar's discovery, that both gp52 and its precursor, pre-gp70, were present in virus-free culture medium from MuMTV-producing cells was unexpected because it was the first

time that a glycoprotein precursor had been found in cell culture medium and because cell surface labelling studies had shown that, unlike MuMTV gp52, pre-gp70 is not present on the surface of MuMTV-producing cells.

Since pre-gp70 is shed into the medium of MuMTV-producing tissue culture cells, yet is apparently not available for surface labelling, two populations of pre-gp70 may exist. One population, destined to provide mature proteins for MuMTV assembly, may be cleaved before reaching the cell surface to produce gp52. The gp52 is then inserted into the cell membrane and leaves the cell only when it is incorporated into a virus particle. The second population of pre-gp70 molecules are shed from the cell without ever becoming integrated into its surface membrane. In that context, it is interesting to note that pre-gp70 has not been reported to be packaged within mature MuMTV particles.

The anti-gp52 serum that Ritzi used (*Proc. natn. Acad. Sci. U.S.A.* **73**, 4190; 1976) reacts with both gp52 and pre-gp70 and thus the glycoprotein that was detected in the plasma of tumour-bearing mice might have consisted of gp52 alone, gp52 and pre-gp70 or even pre-gp70 alone. The discovery that pre-gp70 is shed from mammary tumour cells in tissue culture could therefore explain the observation of Ritzi *et al.* but the mechanism by which MuMTV glycoproteins are shed from cells and the biological significance of shedding are still unknown. However, one can reasonably speculate that shedding helps MuMTV-producing tumour cells to escape from the immunological defences of the host. Immunisation studies have shown that gp52 is the most potent immunogen for protecting mice from exogenous MuMTV infection and subsequent tumorigenesis (Sarkar & Moore *Cancer Res.* **38**, 1468; 1978). It is therefore possible that the release of large quantities of gp52, and possibly pre-gp70, into the plasma provides a means of complexing (and thus, in effect, neutralising) cytotoxic antibodies against the glycoprotein, thereby increasing the survival advantage of MuMTV-producing tumour cells.

Whatever the biological function of glycoprotein shedding, it provides a unique opportunity to investigate two apparently different pathways of pre-gp70 transport and/or processing. It is still not known if MuMTV-producing normal mammary gland cells cultured *in vitro* would shed viral glycoproteins. If it is found that such normal cells do not exhibit the shedding phenomenon, membrane changes taking place in the transition from normal to tumour cells might be studied using MuMTV glycoprotein shedding as a marker for cell transformation. □

Versatile accelerators

from R. G. P. Voss

THE range of uses to which small accelerators are now put is astonishing. These were covered exhaustively in a recent conference in Texas on the application of small accelerators*. The word 'small' is perhaps misleading: there were progress reports on the two very large tandems being built in the United Kingdom and in the United States, at Daresbury and Oak Ridge respectively, and experiments carried out on the 800 MeV proton linear accelerator at LAMPF and the 20 GeV electron linear accelerator at SLAC were reported. Perhaps, as someone remarked, even small accelerators are big in Texas.

Amongst the many different fields, one topic inevitably attracted considerable interest: the application of accelerators to medical purposes. Several papers dealt with the production of radioisotopes by cyclotrons, tandems, and linear accelerators. Information was given on targets, yields, the subsequent radiochemistry, the various uses of the isotopes, and instrumentation to detect localised radioactivity in the human body. Considerable work has been done in recent years at Los Alamos with pions produced by the proton linear accelerator. This is a huge machine, 850 m long, and its suitability for widespread use in medical work is obviously restricted. As a consequence a smaller accelerator, a mere 120 m long, is now being developed. It is known as PIGMI (Pion Generator for Medical Irradiations) and is now in its third year of development, with emphasis on being less expensive (\$10M) and more reliable than accelerators for pure research.

A related field of great interest is that of computed tomography, and developments following from the original EMI CT scanner were presented. Work on proton CT at Los Alamos was described by W. F. Hanson (Oak Ridge Associated Universities) and R. R. Highfill and M. E. Phelps (University of California at Los Angeles) gave a paper on positron tomography. By detecting the annihilation radiation from positrons one can make an *in vivo* study of physiological processes in specific regions of the body. The need for positron-emitting radioisotopes (such as ^{11}C , ^{13}N , ^{15}O , ^{18}F) with short half-lives means that

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accelerators must be available very close to the medical centres.

Two papers dealt with a subject of great topical interest. K. H. Purser (General Ionex Corporation) reviewed the use of electrostatic accelerators to give ultrasensitive particle identification, resulting in a spectrometer which is sensitive to a few tens of particles in a background of 10^{18} . By stripping electrons after an initial acceleration, one dissociates molecules and so enormously simplifies the identification of the atoms one is looking for. These need not be radioactive isotopes (as was necessary, for instance, in the previous method of counting ^{14}C decays): it is now possible for instance to study Li, Mg, Si and Cl. Moreover, the rare earths form excellent tracers. Extensions to the technique are leading to a new generation of secondary ion mass spectrometers many orders of magnitude more sensitive than earlier ones. H. E. Gove (University of Rochester) reviewed progress in ^{14}C dating in the remarkable 18 months since the technique of using an electrostatic accelerator was first reported. It is possible to date back to 70,000 years or even earlier, and a few milligrams of sample are sufficient (Doucas *et al.* *Nature* **276**, 253; 1978 and Hedges & Moore *Nature* **276**, 255; 1978). The technique is being constantly extended. Work has been done on ^{36}Cl for instance, for dating old ground water with a view to the safe storage of nuclear waste (as reported in this issue of *Nature* on page 22) and on ^{10}Be for 'million year geochronology'.

A number of papers dealt with various aspects of thermonuclear reactions. D. J. Nagel (US Naval Research Laboratory) discussed the progression from laser light through electrons to light ions and then to heavy ions in developments aimed at thermonuclear fusion. Currents of 300 kA of protons of energy about 1 MeV can be produced, with substantial fractions of a terawatt in a few nanoseconds. Even higher pulse energies are planned using 25 GeV uranium ions. Intense ion beams on solid targets will produce and heat plasmas to temperatures of the order of 10^5 K. He also described fascinating work on multi-atom projectiles: diamond dust is levitated while charge is built up with, for instance, a proton beam. Then the dust particle is accelerated by a 2 MV Van de Graaff accelerator. Twenty such particles can be accelerated per day! These are then fired into solid targets, to get plasmas with temperatures of the order of 10^4 K.

Work on new uses of polarised beams was presented by S. S. Hanna (Stanford University). A polarised beam is implanted in a target, and if this has the appropriate properties the polarisation transferred to the nuclei of the target can be preserved. This serves as a probe for studying magnetic moments, quadrupole moments (if an electric field is present), hyperfine fields, lattice effects, and for solid state problems such as the relaxation time of an implanted nucleus in a holding material. Polarised beams are being used as gas targets, thus enabling polarised-polarised reactions to be studied.

S. Raman (Oak Ridge National Laboratory) reported on a search for superheavy elements in giant-halo monazite inclusions. Use was made of the particular advantages of synchrotron radiation (from the electron storage ring SPEAR at Stanford) to

induce fluorescence X rays. No sign of superheavies was detected.

Many other topics were covered: geoscience and mineral exploration, neutron activation (with applications to agriculture, archaeology, the life sciences), trace element analysis, radiation processing of foods and sterilisation of medical products, the curing of paint, the crosslinking of polymer insulating and packaging material. Papers on ion implantation covered amongst other things the processing of semiconductors, integrated circuits, metal corrosion, and a project for an implantation for silicon solar cell processing (P. H. Rose, Nova Associates) with a capacity of about one acre per day!

The overall impression of the conference was the enormous and rapidly growing use of accelerators in an extraordinary range of applications. □

Semliki forest virus: key to the MHC?

from Polly Matzinger

IMMUNOLOGISTS have long been intrigued and biochemists frustrated by the cluster of genes collectively known as the major histocompatibility complex (MHC). To immunologists it is a fascinating bit of chromosome. Cell surface molecules coded by genes in the MHC have a central role in the processes by which the immune system recognises self from non-self (see *News and Views* **272**, 112; **274**, 840; 1978;). They are involved in the recognition processes which lead to graft rejection, immunity against infectious agents, susceptibility to a wide range of diseases, and have even been shown to affect mating preferences in mice (*Immunogenetics* **6**, 265; 1978). Consequently, many theories and much of the current work in immunology concern the MHC.

The MHC has been difficult to study biochemically. Purification and characterisation of its products has been an arduous task replete with surprises. In 1956 the mouse MHC, H-2, was thought to be DNA. In 1957 it was a carbohydrate and by 1966 it had turned into a lipid. Today it is a glycoprotein with the arguments still raging over the antigenic importance of the carbohydrate parts (Parish *Immunogenetics* **6**, 343; 1978). A major cause of frustration and confusion has been the difficulty of purifying MHC products in sufficiently large quantities; however, a recent article from Helenius *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3846; 1978) promises an easier road ahead.

Ari Helenius and his colleagues

report that Semliki Forest Virus binds preferentially to MHC products on the cell surface. They discovered this while testing binding properties of the virus. They were using antibodies directed against Semliki Forest Virus to detect its binding to cells and antibodies directed against H-2 as a control. To their surprise they found that target cells which had been incubated with Semliki Forest Virus would no longer bind anti-H-2 antibodies. They postulated that the virus was binding to H-2 and therefore blocking the H-2 molecules from the anti-H-2 antibodies. Further tests showed that Semliki Forest Virus also bound to human cells bearing HLA molecules (the human MHC) but not to their mutant counterparts lacking HLA. Moreover, the virus binds to artificial lipid vesicles which have had MHC products inserted and can even be used on a Sepharose column to purify MHC molecules from solubilised membranes. So Semliki Forest Virus should make the purification of MHC molecules much simpler.

Like protein A from *Staphylococcus*, which is useful in the purification of antibody molecules, Semliki Forest Virus has another interesting use. Protein A, which binds to the Fc part of antibodies has been used in phylogenetic screens to study the evolution of antibodies (Kronval *et al.* *J. Immun.* **104**, 140; 1970). Semliki Forest Virus may do the same for the MHC. There

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has been much speculation about the evolution of the MHC. Simple self-non-self discrimination is found in very primitive organisms. Sponges only fuse with genetically similar sponges and, in colonial ascidians, recognition of the products of a single locus determines whether free swimming tadpoles will join up to form colonies. Even mating preference is controlled by this locus in ascidians. It has been postulated that the MHC may have evolved from genes that control such primitive self recognition and that our immunological recognition system is simply a more sophisticated version of that of amoebae and sponges (Langman *Rev. Physiol. Biochem. Pharm.* **81**, 1; 1978; Lafferty & Woolnough, *Immun. Rev.* **35**, 231; 1977; Burnett & Fenner *The Production of Antibodies*, Cambridge University Press, 1949). So far such speculation has chiefly fuelled fire-side debates, but now Semliki Forest Virus could be used to test these theories.

The virus has an amazingly wide host range, having been grown in insect as well as avian and mammalian cells. To infect such a wide array of cells it must either have a wide range of cell surface receptors or a receptor which is evolutionarily highly conserved. A hint

that the receptor is conserved comes from Helenius *et al.*'s paper showing that the virus binds both human and mouse MHC molecules. Will it bind to primitive organisms? We may soon have an answer from Virginia Scofield and Irv Weissman at Stanford who are testing its binding to the colonial ascidian *Botryllus schlosseri*. Binding *per se* is no proof that the molecules are evolutionarily related, but Lee Hood's group at CalTech may have a solution to that problem. They will soon be able to sequence picomolar quantities of protein at the rate of 40–60 residues a day. It will then be entirely possible to isolate a Semliki Forest Virus binding protein from a small number of *Botryllus* (say one seaweed leaf's worth) and sequence it to see how it compares with the evolutionarily more advanced MHC molecules.

The story is told of an explorer who, coming on a forest not marked on his maps of Africa, asked his native guides what it was called. 'Semliki', they said, shaking their heads, 'Semliki'. Little did he know when he named it Semliki that Semliki means 'I don't know'. Ironically the virus named Semliki may well fill in some of the gaps in our knowledge of the elusive MHC. □

Storing energy as acid

from A. B. Hart

WHEN everybody had a woodpile, a coalshed or an oiltank there was little fuss about matching the rate of production of an energy source to the consumer's need. But for the consumer with a taste for the convenience and cleanness of electricity in his domestic or industrial premises it is another matter. Although electricity plants can lay in stocks of coal, oil or uranium the plants themselves are costly and complex and it is both financially and mechanically unsound to turn them down as the demand for electricity falls back in its diurnal or seasonal cycle. Indirect storage of domestic electricity is possible in off-peak space heating and in the future perhaps in electric battery motor cars. This kind of consumer 'storage' may well play a large part in the future planning of a power network, but there will remain a need for a balancing store near the generators and under the control of the operators of the network, so that the plant can be kept running at a steady load.

In recent years true storage has been available using hydroelectric plant in reverse; storing electrical energy as

potential energy of water. But hydroelectric plants are usually expensive to build and in the UK there are going to be few sites left to meet the demand for storing night-time electricity expected around the turn of the century. This is when the proportion of electricity generated by nuclear power (not to mention wind and wave power with their unavoidably intermittent characteristics, which call for a very large storage element) is likely to be increasing rapidly.

Other methods of energy storage have recently attracted much interest. Flywheels, superconducting magnets, compressed air and heat, underground water reservoirs, and rechargeable electrochemical batteries are all under scrutiny (Gardner *et al. CEEB Research* **2**, 1975). One of the more radical ideas is to make hydrogen from spare electricity by electrolysis of water or directly from nuclear heat. The hydrogen need not be entirely reconverted to electricity; it can be used as a chemical feedstock—for fertili-

sers, petrochemicals and for hydrogenating coal to provide liquid fuels. This is an introduction to the much broader idea of the 'hydrogen economy' (Getoff *Wasserstoff als Energieträger*, Springer Verlag, Vienna 1978) a scenario in which all primary energy sources old and new, fossil, nuclear, solar, winds, waves and biomass, are converted into hydrogen which is stored as liquid or gas and piped to the consumer for his total energy needs. Objective attempts to make an economic case for such an all-embracing hydrogen energy system depend very much on the cost, efficiency and safety of storing the hydrogen. For fuelling transport one could use liquid hydrogen or a metallic hydride, neither of them cheap nor particularly attractive, the former because of the cost of the container, the latter because of the heavy weight of the metal. For storage in large-scale power networks one thinks of the gas in high pressurised pipe-lines or underground caverns. All feasible but somehow a little unnerving; and the feeling remains that storage and transport is rather a weak point in the idea of the hydrogen economy.

A recent paper from the RCA Laboratories at Princeton in the US (Williams, Crandall & Bloom *Appl. Phys.* **33**(5) 381; 1978) suggests an interesting alternative. Linking the hydrogen to CO₂ to give formic acid, HCOOH, from which it can be released using a palladium catalyst, one obtains a stable liquid energy storage medium, equivalent, even with the formic acid as a 4 mol l⁻¹ (4 M) aqueous solution, to the gas held at 100 atm pressure in a heavy cylinder. The acid can be made electrolytically in the 4 M solution by the reduction of CO₂ (or bicarbonate ions, HCO₃⁻) with oxygen appearing at the other electrode. Using an amalgamated nickel electrode for the CO₂ reduction with an ion exchange membrane to separate it from the oxygen electrode the authors observe only an extremely small current (1 mA cm⁻²) even with a high overpotential (1.0 V) denoting a very low electric energy to hydrogen conversion efficiency and a low specific output—which implies a very large investment in electrolysis plant. On such a basis the conversion cost would be prohibitive even without taking account of the palladium hydrogen release catalyst. And that is not cheap; a simple calculation suggests over £3,000 worth of palladium-carbon at current prices to liberate enough hydrogen to power a small motor car (3.5 kW electrical output). But such calculations are mischievous—the authors have made a laboratory demonstration of the process, not a pilot plant demonstration unit.

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It is the basic idea of going directly from electricity to a stable liquid energy storage medium which is so exciting. Further work must examine what energy losses are inevitable in the formic acid system to see whether they can be accepted and whether other losses can be avoided at acceptable cost. Electrochemical studies must also show whether by working under different conditions, for example, at higher temperatures and pressures, one could achieve a higher concentration of the acid (a 60% solution would contain more than half as much hydrogen on a volume basis as liquid hydrogen itself). It is by no means evident that the electrolytic liberation of hydrogen atoms in close proximity to CO₂ molecules, leading to the formation of HCOOH, should not at a suitable surface, proceed as efficiently at a given surface-specific rate as in the absence of CO₂. In this case the atoms form hydrogen molecules, as in the electrolysis of water to form hydrogen gas at the cathode. And advanced electrolysis of water can achieve a high (75–90%) efficiency of conversion of electrical

energy into hydrogen (expressed as the heat of combustion to water), vastly higher than the authors' experiments seem to suggest for formic acid formation. A review of the electrochemical literature (Russell *et al.* *J. Electrochem. Soc.* **134**, 1329; 1978) suggests that the authors' low efficiencies and low specific rates of formic acid formation are expected for this type of electrode process, where the hydrogen atom must be liberated at a high over-voltage (also with very poor electrical efficiency) to be able to break open the CO₂ molecule and react with it. One might get over this problem but it is apparent that considerable research is needed.

Meanwhile, it may certainly be reasonable for the authors to claim, as they do, that the system "represents an energy storage method of exceptional versatility and generality" but until much more work is done it is surely rather over-optimistic to state that it "will be important for the storage of electrical energy whenever there are surpluses". □

New Zealand the pathogen was readily graft transmitted and some field spread has been recorded (*N.Z.J. agric. Res.* **14**, 735; 1971; *A. J. R. N. Z. Inst. Hort.* (2), 16; 1974). In California rose spring dwarf (*Pl. Dis. Repr.* **55**, 294; 1971) was reported and described as a graft transmissible disease distinct from rose wilt (*Pl. Dis. Repr.* **60**, 183; 1976). In addition rose leaf curl, a distinct component of a disease complex which resembles rose wilt, has also been described in California (*Pl. Dis. Repr.* **60**, 178; 1976). The question therefore remains of what is rose wilt and which pathogens cause it. Poor graft union formation, stunting and proliferation are disorders that can be induced by many pathogens, physiological conditions and environmental factors in woody plants. In view of this, to suggest endemic A2 pathogenic causes and to recommend quarantine procedures on that basis is both naive and foolhardy. Quarantine procedures should be based on the aetiology of a disease, not on disease expression.

The EPPO Working Party also seems to be rather inconsistent in its recommendations for the control of A1 pathogens. For the control of needle cast of Japanese larch caused by *Mycosphaerella larici-leptolepis* they state that "the importation of larch from Japan should be prohibited". However, in respect of American plum line pattern virus they say that it is doubtful whether roguing of trees should be recommended. This contradicts the original recommendations for A1 organisms of taking necessary and reasonable action to prevent entry.

The lists of A1 and A2 pathogenic organisms are abysmally short when one considers the vast numbers of economically important pathogens that have been described throughout the world. It is to be hoped that EPPO will rapidly extend their lists and that the data sheets will provide more comprehensive and accurate information on these pathogenic organisms.

Quarantine measures can only be effective if those responsible for them are well informed. Bureaucrats with little understanding of plant protection must seek the guidance of leading research workers otherwise confusion will result.

Non-endemic pests and pathogens are being imported to Britain annually, and the situation in continental Europe is even worse. EPPO's function is to advise on the restriction of movement of pathogenic organisms into and within Europe without impairing plant trade. Let us hope that it will adopt the correct policies to fulfil this role and not imitate the Plant Health Branch of our own Ministry of Agriculture, Fisheries and Food (see *Nature* **259**, 449; 1976).

European plant health

from a Correspondent

THE introduction of plant pathogens into a country in which they are not normally present can have devastating economic or environmental consequences. To prevent the inadvertent introduction of serious pathogens, stringent and well-informed quarantine regulations are necessary. New quarantine measures for continental Europe and Britain have been proposed by the European and Mediterranean Plant Protection Organisation (EPPO *Bull.* special issue 1975) and the EPPO have recently published the first set of data sheets on which these recommendations are based (EPPO *Bull.* **8**(2); 1978). These data sheets list some of the organisms covered by the quarantine regulations which are classified as A1 (organisms not present in the EPPO area) and A2 (present in the EPPO area). They provide a description of the pathogens and some of the diseases they cause.

At first glance this appears a commendable piece of work, but is it? One deficiency, which has caused widespread concern amongst plant pathologists who have the task of interpreting the regulations is that variations in strain, race and serotype are ignored. The potential importance of such differences can be seen in British hedgerows, now lined with rows of dead elms as the result of the introduction

of a variant strain of a pathogen already classified as endemic within Europe (including Britain).

With regard to the diseases with a viral aetiology the picture is confused by the lack of any reference to a definitive description of the virus such as the AAB/Commonwealth Mycological Institute Descriptions of Plant Viruses, and by the omission of any description of the morphology, composition, grouping or serological grouping of the virus. How then is anyone to know exactly which virus is being referred to and in that case how can "an EPPO country take whatever action is necessary and reasonable to prevent introduction" (EPPO *Bull.* 1975 special issue).

Plant virus nomenclature is notoriously confused by the practice of naming the viruses after the disease they cause and it seems a shame that the EPPO have not learnt this lesson. For example they describe Scottish raspberry leaf curl as a 'completely different disease' from American raspberry leaf curl and attribute rose wilt syndromes to endemic A2 pathogen(s) when plainly this is unlikely to be the case.

In Britain and Holland no clear evidence that rose wilt or dieback was graft transmissible has been found (*Phytopath. Z.* **79**, 160; 1974; *Neth. J. Pl. Path.* **81**, 187; 1975); whereas in

review article

Present status of γ -ray astronomy

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Diffuse extragalactic γ -ray flux may be due to individual objects, and two possible sources are 3C273 and NGC4151. The Milky Way is an intense line source exhibiting a large contrast in emissivity between the directions towards and away from the galactic centre, respectively. γ -ray point sources and cosmic rays interacting with interstellar material are both responsible for this emission—the latter decreasing away from the centre. Twenty-one point sources have been discovered to date, including four pulsars which all have two maxima with ~ 0.4 phase separation. If the number and position of γ -ray point sources are proportional to the stellar birth rate, the bulk of the galactic emission can then be explained by 5,000 sources emitting the 10^{42} photons > 100 MeV s^{-1} produced in the galaxy.

THE new discoveries by ESA's satellite Cos B (refs 1–3) and successful⁴ balloon observations provide new material for a discussion of the astrophysical processes leading to γ -ray production. The sky in the light of γ rays shows two distinct features, a general and rather diffuse flux from extragalactic space against which the narrow and brilliantly luminous band of the Milky Way stands out.

γ Rays from extragalactic space

The cosmos is filled with photons of all wavelengths. If one considers an average point in intergalactic space, the energy density of photons in the various energy bands is like that shown in the hatched area⁵ of Fig. 1; the full line represents the energy density in photons from the Galaxy in the solar neighbourhood. Remarkably the energy density in metagalactic space remains at the high level of several 10^{-5} eV cm^{-3} in the X- and γ -ray domain. This is because hard X rays and γ rays are practically not absorbed⁶ (the redshift, at which the cosmos becomes opaque to γ rays is $z \sim 100$) and distant sources therefore make a similar contribution to the diffuse flux as close ones. Thus, extragalactic γ -ray astronomy must be considered with cosmological questions.

Observationally, there seems to be agreement about the shape of the energy spectrum of the diffuse radiation^{7–9}. Figure 2 shows a shoulder at around 3 MeV which steeply drops beyond 10 MeV. The data at lower energies accord well with the results at high (> 50 MeV) energies, if allowance is made at high energies for a flat galactic contribution to the extragalactic flux.

The origin of the diffuse radiation in general and the shoulder at 3 MeV in particular is still unclear; is it due to a truly diffuse mechanism, or to an as yet unresolved superposition of many individual sources? We encounter the same problem when we consider the origin of galactic γ rays.

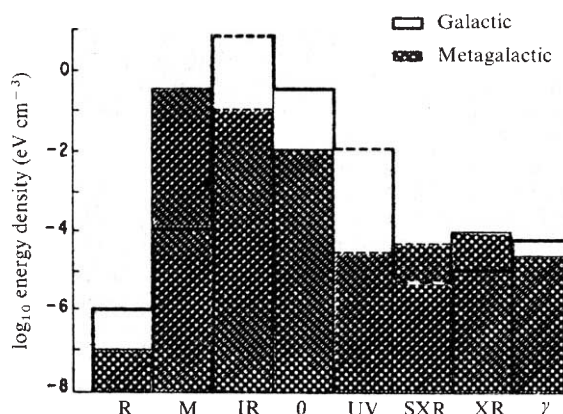
This uncertainty has been due to, on the one hand, the little observational evidence available on the γ -ray luminosity of galaxies, quasars and other objects, and on the other hand, the models for truly diffuse production either in intergalactic space now or during the big bang have free parameters. It has thus been possible to reproduce the same observational results with several different models. For example, the diffuse radiation could originate from electrons that escape from galaxies into intergalactic space, or at least the shoulder could be caused by

redshifted photons from π^0 -decay that in turn have been produced either by a copious supply of cosmic rays in the big bang, or by simultaneous baryon-antibaryon annihilation^{5,10}.

Some new light has been shed on this situation by the observations of two extragalactic γ -ray sources^{3,4} which have been identified to be 3C273 and the Seyfert galaxy NGC4151. If these identifications are correct, 3C273 would emit 2×10^{46} ergs $^{-1}$ in 50–500 MeV γ rays, and the spectrum of NGC4151 would look like that shown in Fig. 3. Certain assumptions about space density and luminosity, the diffuse γ ray background could then be explained by a superimposition of individual sources⁴.

These new discoveries have directed attention towards extragalactic γ ray astronomy for other reasons. One possible mechanism for producing the sharp cut-off in the spectrum of NGC4151 at 3 MeV is the Compton process¹¹. Due to large energy losses, a sharp cut-off will have been generated in the energy spectrum of the electrons required to scatter photons into the γ -ray domain which then reproduces itself in the spectrum of NGC4151. If this were true, the position and shape

Fig. 1 Energy density of the cosmic photons. The hatched area shows the energy density of photons in the various energy bands (radio, R; microwave, M; infrared, IR; optical, O; ultraviolet, UV; soft X ray, SXR; X ray, XR; and γ ray band) in metagalactic space; the full line shows the galactic contribution evaluated in the solar neighbourhood. After Silk⁵.



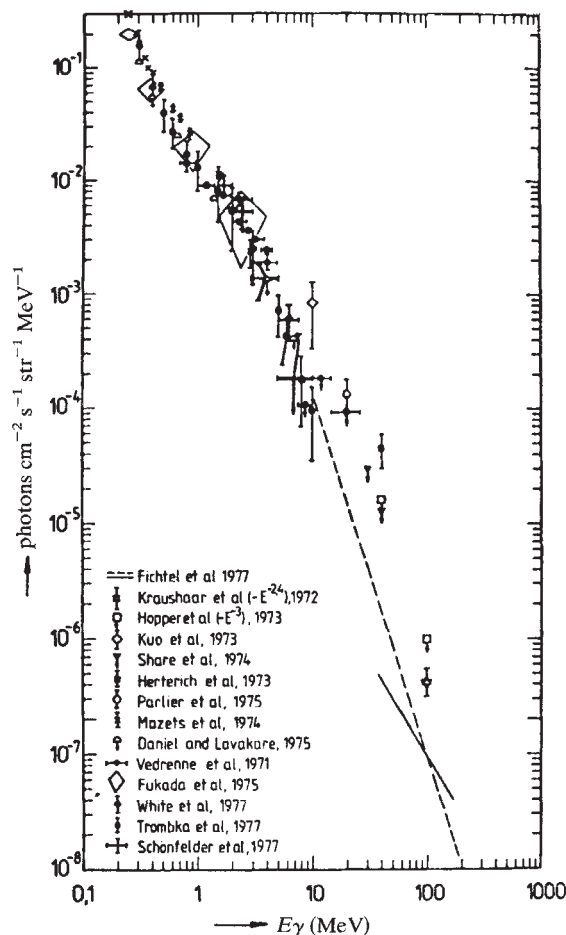


Fig. 2 Energy spectrum of the diffuse γ radiation after Schönfelder *et al.*⁷, Fichtel *et al.*⁸ Taken from ref. 9. The solid line is the contribution of galactic γ rays to the total diffuse flux from high galactic latitudes, the dashed line is the remaining extragalactic flux if the galactic contribution is subtracted from the total diffuse spectrum.

of the cut-off must be highly time-variable¹² and it would give some indication of the energy requirement and rate of occurrence of events that power Seyfert galaxies like NGC4151.

γ Rays from the Galaxy

The galaxy appears as a narrow line source in high-energy γ rays, its width at half maximum¹³ is $+3^\circ \geq b^{\text{II}} \geq -2.5^\circ$ at $l^{\text{II}} = 0^\circ$, and $+7^\circ \geq b^{\text{II}} \geq -3^\circ$ at $l^{\text{II}} = 120^\circ$. (The significance of this deviation from the galactic plane would be interesting to consider.)

Quantitatively, the line source strength of the galaxy is shown¹³ in Fig. 4. This is an old picture: although the galactic survey has been completed by Cos B, but the new data have not been published yet. However, in the complete data set, regions of intense γ -ray emissivity in the plane seem to occur when the line of sight tangentially intersects spiral arms which in turn have been localised by high excitation parameter H II regions and intensity maxima in the radio continuum and neutral hydrogen¹⁶.

γ -ray point sources are also embedded in the galactic plane. Their positions (and identifications, if any) are indicated by arrows in Fig. 4. A list of point sources is given in Table 1 (refs 17, 18). The nature of the galactic γ -ray emission has been widely debated. γ rays originate from the interactions of cosmic-ray nucleons and electrons with interstellar matter, yet the observed line source strength shown in Fig. 4 in the direction towards the centre differs strongly from the fluxes expected in a simple model where cosmic rays (with the average galactic intensity as observed near the Solar System) interact with inter-

stellar atomic hydrogen. These expected fluxes would not vary much as a function of longitude, because the projected density of atomic hydrogen along the line of sight also is rather constant. In this simple model, the γ -ray line intensity over the entire longitude range would be that indicated by the dashed lines in Fig. 4. Clearly, many more γ rays come from the longitude interval between $\pm 60^\circ$ than can be explained by this simple model¹⁹.

One way of explaining this discrepancy would be to assume that more material and cosmic rays are available in the inner parts of the Galaxy. On the other hand, γ rays also originate in point sources. Can they account for the difference? The discussion is complicated by the fact that the angular resolution of a γ -ray telescope (several degrees) is insufficient to directly answer these questions.

Discrete sources

The truly diffuse component is that part of the galactic radiation that remains if point sources are subtracted. It is therefore necessary to consider these first.

γ -ray point sources are of high astrophysical interest in their own right and indeed the impact of γ -ray astronomy on the

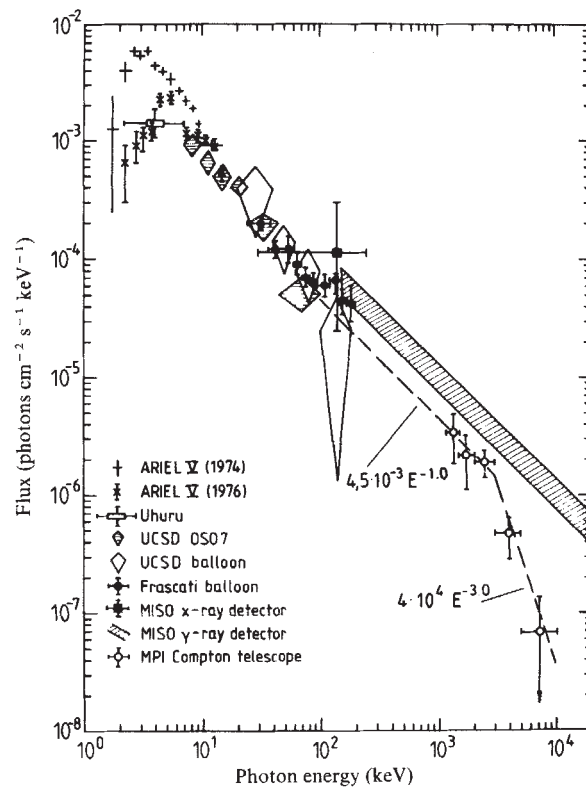


Fig. 3 The energy spectrum of γ rays from the direction of NGC4151. After Schönfelder⁴. The MISO γ -ray detector actually measured an integral flux > 150 keV and not a spectrum. The hatched area represents the spectrum derived by these authors, and compatible with their integral flux under the assumption of an $E^{-1.0}$ power law extending to infinity. It thus does not conflict with the measurements of the MPI Compton telescope.

whole of astrophysics may eventually be larger here than in the study of the regions of residence of cosmic rays in the Galaxy.

Due to the limited angular resolution, point source identifications in the Galaxy have been possible only for pulsars^{20,21}, the number of which has now been increased due to Cos B to be four²²⁻²⁴: PSR1822-09, PSR0531+21, PSR0740+28 and PSR0833-45. It is very surprising that all four pulsars show double maxima with a phase separation of 0.4 (Fig. 5), although their energy spectra are different.

Furthermore, the luminosities of the four pulsars (using the dispersion measure as distance indicator) do not decrease in

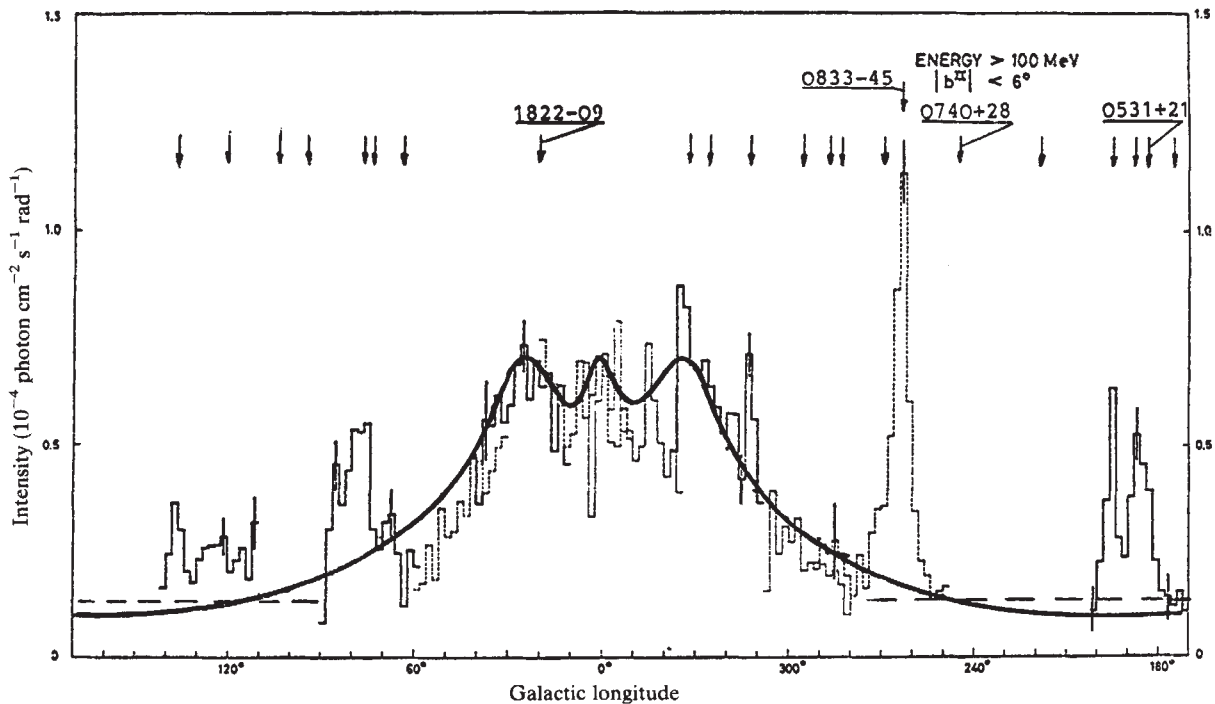


Fig. 4 Longitude profile of the intensity of γ rays of energy >100 MeV over the latitude range $|b^{\text{II}}| < 6^\circ$. The dashed line roughly indicates the line intensity expected from cosmic rays interacting with interstellar gas in the region $l^{\text{II}} = 180^\circ \pm 80^\circ$ after Fichtel *et al.*¹⁴, allowing for the narrower latitude range $|b^{\text{II}}| < 6^\circ$. The solid line is the distribution in apparent brightness of O-stars as given by their Lyman-continuum flux after Mezger¹⁵; it has been normalised to the average line intensity of $\sim 10^{-5}$ photons >100 MeV $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ contributed by the Cos B point sources from the two viewing periods centred on 126° and 185° , respectively.

proportion to decreasing $\dot{P}/P^3$ which in turn is proportional to $\dot{E}$, the rate of loss of rotational energy (assuming the same momentum of inertia in all four pulsars). Thus, at least in the four cases observed (which may be subject to selection effects), the efficiency of converting $\dot{E}$ into γ -ray luminosity increases with decreasing $\dot{E}$ until some as yet unknown limit is reached²³⁻²⁵.

Finally, at least in the case of the Crab (0531+21) and Vela (0833-45), the emission from these sources is compatible with it being entirely pulsed. The 2σ lower limit of the pulsed fraction is 78% (>50 MeV, Crab) and 91% (>50 MeV, Vela), respectively²².

Attempts have been made to identify at least some of the Cos B sources with other objects¹⁷ like X-ray sources^{26,27}, radio-optical sources, supernova remnants²⁸, H II-regions²⁹, and dark clouds³⁰ (which radiate γ rays if they are penetrated by cosmic rays). Due to the large positional error in the γ ray observations, these have remained possibilities.

General galactic emission

In the outer parts of the Galaxy, γ rays are clearly produced by both, cosmic ray interactions with interstellar matter, and point sources. A cosmic-ray gradient away from the galactic centre must exist⁴³, because the γ -ray intensity seen from the outer parts is less than that expected if cosmic rays were to fill the entire galactic disk with the same intensity as that near the Solar System.

Whether or not the high flux value of γ rays from the inner parts of the galaxy is due to point sources or a truly diffuse process requiring more interstellar material and/or more cosmic rays there, is as old a question as the first positive results of γ -ray astronomy^{19,31}.

It has been shown¹⁹ that the radio emissivity, the distribution of neutral and molecular^{32,33} hydrogen alone do not sufficiently well represent the spatial distribution of general γ -ray emissivity in the Galaxy. Consequently, one has to construct the population of γ ray emissivity theoretically by assuming a correlated

variation of cosmic-ray energy with matter density, be it in the spiral arms¹⁴, or in a ring of high matter density as provided by molecular and atomic hydrogen³³.

These models of the galactic γ -ray emissivity have been constructed so that they can explain quite well the observational results. But they are not conclusive because the actual amount of interstellar material available as an interaction partner for cosmic rays, and the actual intensity of cosmic rays throughout the galactic plane are uncertain. Therefore there is still the possibility that the observed picture of our Galaxy in high energy rays could be dominated by point sources, γ -ray stars, rather than diffuse processes. Because individual γ ray sources cannot be resolved in an environment of large emissivity, and time variability may help identification in many but probably not all cases, many statistical arguments have been used with conflicting results. For example, Ögelman³⁴ attempted to explain the OSO 3 results by extrapolating the intensity of known X-ray sources into the γ -ray domain using a -2.0 power law index; the uncertainty of such a method was pointed out by Clark *et al.*¹⁹. Similarly, Coe *et al.*²⁶ extrapolated the apparent brightness of X-ray sources into the γ -ray domain, using the 4th Uhuru catalogue and Ariel 5 observations. They used their results to calculate an average for the galactic γ -ray emission by point sources, while Protheroe *et al.*³⁵ came to the conflicting conclusion that γ -ray point sources, based on a comparison with the 4th Uhuru catalogue, could account only for 19% of the galactic line emission.

The situation is also unclear if pulsars are used to estimate the possible contribution of point sources. For example, Kniffen *et al.*³⁶ estimated the pulsar contribution to galactic γ rays at 5%, while Higdon *et al.*³⁷ believe that a much larger contribution could be expected.

The problem with statistical arguments is that a known population is required, that has the same spatial distribution within the galaxy as that expected for the γ -ray sources. It has not been shown that the X-ray sources provide such a population. There are indications that the distribution of pulsars in the galactic plane³⁸ may be quite similar to the γ -ray emissivity.

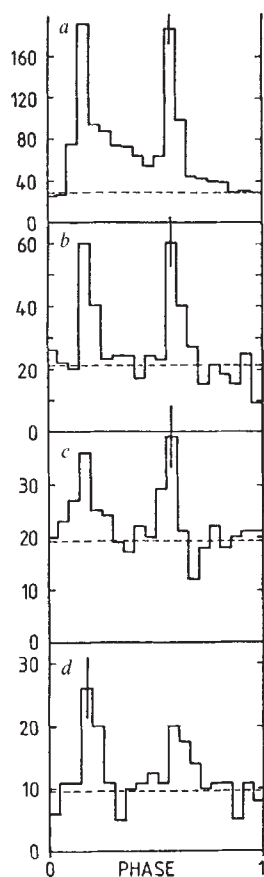


Fig. 5 Light curves of the four pulsars now observed by Cos B (refs 19–21). *a*, PSR0833–45; *b*, PSR0531+21; *c*, PSR1822–09; *d*, PSR0740–28.

A statistical investigation of this question may start from the observations of point sources in the anticentre direction, assuming that all have been discovered in favourable conditions of observation, namely low background and low space density of sources which then allows detection in spite of the low power of angular resolution of the γ -ray telescope (several degrees). One then may extrapolate this result to cover the entire galactic plane, scaling it according to the variation of a known population of astronomical objects which are believed to have a similar space dependence as γ -ray sources. Attempts using X-ray sources or pulsars have led to conflicting results.

One may, however, investigate whether γ -ray point sources could be young objects and thus be correlated to the stellar birth rate. It had been noted (see, ref. 41) that the distributions in the galactic disk of H_2 , H II-regions, γ rays and supernova remnants are all similar. However, the question of the stellar birth rate has recently been reviewed^{39,40} and has quantitatively been estimated by counting the number of Lyman continuum photons emitted by O-stars per element of volume of the galactic disk. From observations of giant radio H II-regions³⁹ and the extended low density H II-regions, Mezger¹⁵ was able to derive the emissivity of the galactic disk in Lyman continuum photons as a function of galactocentric distance.

It is proposed here that the spatial distribution of γ -ray localised sources closely resembles that of the O-stars, that is that of the Lyman continuum photons (Fig. 6). The width of that layer in the galactic plane is very narrow, ± 100 pc, in agreement with the narrow width of the galactic γ -ray emissivity.

One may now construct the line source intensity of apparent brightness of the Lyman continuum photons by using the data of Fig. 6 and integrating along the line of sight through the galactic plane, taking account of the limited thickness of the disk: this is the full line of Fig. 4 and it supports the conjecture that the

population of O-stars has the same spatial distribution as the γ -ray emissivity.

Figure 4 shows a rough comparison which is circumstantial in the sense that Mezger¹⁵ unravelled observational results to arrive at the galactic production rate of Lyman continuum photons which then had to be reconverted to the distribution of apparent brightness along the line of sight (Fig. 4). However, a detailed comparison of extended low-density H II- and radio H II-regions with the γ -ray sky map (which will be forthcoming shortly from the Cos B observations) may reveal a much greater agreement in detail. Already, correlations between Cos B point sources and H II-regions have been noted^{29,32}, and the spiral structure, which also is visible in the γ -ray sky surveys, is in turn delineated by H II-regions¹⁶. Also, 74% of the Lyman continuum emissivity is estimated to come from the main spiral arms, yet only 13% from the interarm region, and 13% from the galactic centre³⁹. Thus, if more γ rays are found to come from spiral arms¹⁴, it may be because the γ -ray point sources have a larger density there.

Approximately, then, 3×10^{11} Lyman continuum photons are emitted per single γ ray with energy exceeding 100 MeV, corresponding to a galactic luminosity of 10^{42} (γ 's > 100 MeV s^{-1}). In other words roughly 10^{49} γ rays above 100 MeV are produced per birth of one solar mass.

These considerations may now be applied to an investigation of the relative importance of point sources and diffuse emission.

Table 1 γ -ray sources detected by Cos B

Galactic coordinates l^{II}	b^{II}	No. of observations	Spectral class	Remarks	Relative intensity
21.0	+1.0	1	Soft	PSR1822-09	~ 0.8
65.5	-0.0	3	Hard		$0.30^{+0.10}_{-0.05}$
75.0	+0.0	1	X		$0.45^{+0.15}_{-0.10}$
78.5	+1.5	1	X		$1.00^{+0.30}_{-0.20}$
95.5	+4.5	2	Hard		—
106.0	+1.5	1	X		—
121.0	+3.5	1	Hard		$0.20^{+0.10}_{-0.05}$
135.5	+1.5	2	Medium		$0.30^{+0.15}_{-0.10}$
176.0	-7.0	1	Soft		0.50 ± 0.10
184.5	-5.5	2	Medium	PSR0531+21	1.00
189.0	+1.0	1	X		0.40 ± 0.10
195.5	+4.5	2	Hard		0.90 ± 0.20
219.0	-0.5	1	Medium		—
243.0	-2.5	1	Hard	PSR0740+28	~ 0.4
263.5	-2.5	5	Hard	PSR0833-45	3.30 ± 0.50
270.0	-1.0	2	Soft		—
284.0	-1.0	1	Hard		—
288.5	-0.5	1	Medium		—
291.0	+65.0	1	Medium	3C273	~ 0.25
295.5	+0.5	1	Medium		—
312.0	-1.5	1	X		0.45 ± 0.15
327.5	-0.5	1	X		0.35 ± 0.15
333.5	+0.0	1	X		0.70 ± 0.20
353.0	+16.0	1	Hard	in Ophiuchus dark cloud complex	

The information is preliminary; analysis continues. Error circles for positions have radii between 0.5° and 2.0° . With the exception of CG263-2 source intensities (> 100 MeV) are within the range 0.2 to 1.0 times that of CG185-5. Spectral clarification is based on the relative numbers of photons detected in the ranges 50 to 150 MeV and > 150 MeV. The pulsar identifications are confirmed by timing phase analysis. Sources marked X are located in complex regions where more detailed analysis is needed to provide information additional to that given in the first Cos B catalogue.

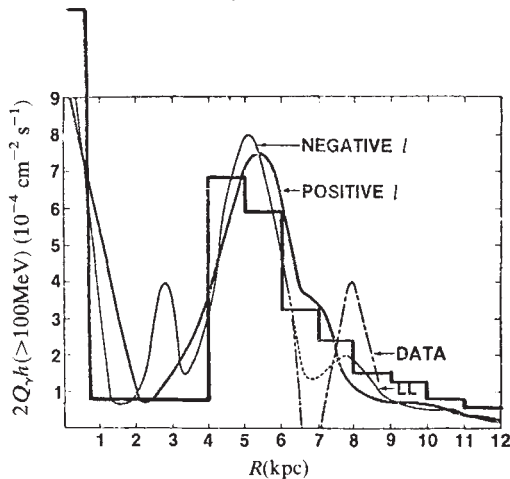


Fig. 6 Modified from Stecker³², the smooth lines being the result of an unfolding of γ -ray line intensity data assuming cylindrical symmetry of γ -ray emissivity in each half of the galaxy. (Compare also Strong *et al.*⁴²). The histogram represents the source function of Lyman continuum photons after Mezger¹⁵, if 3×10^{11} Lyman continuum photons are produced per γ ray > 100 MeV.

Figure 4 shows that the Lyman continuum population has nearly the same apparent brightness for the longitude interval $l^{\text{II}} = 180^\circ \pm 80^\circ$. Two Cos B observation periods¹⁷ centred at $l^{\text{II}} = 126^\circ$ and 185° fall within that longitude. During each viewing period, an angular interval of ~ 0.6 rad in longitude was observed.

There are six point sources with, five without the Crab in both angular intervals (including one source at $b^{\text{II}} = 7^\circ$), leading to a contribution of point sources of 1.4×10^{-5} photons > 100 MeV $\text{cm}^{-2} \text{s}^{-1}$ with, and 10^{-5} photons > 100 MeV $\text{cm}^{-2} \text{s}^{-1}$ without the Crab. In both regions, an angular width of ~ 1.2 rad was scanned, thus the line source contribution of point sources is $\sim 10^{-5}$ photons > 100 MeV $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$.

One may now scale the number of γ -ray point sources and their apparent brightness according to the stellar birth rate, that is the apparent brightness of O-stars as given by the solid line of Fig. 4. This line has been normalised to the value of $\sim 10^{-5}$ photons > 100 MeV $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ at the two viewing directions of Cos B used above. Figure 4 shows that the flux of these point sources can fully explain the shape and enhancement of the γ -ray line source towards the galactic centre.

On the other hand, one may argue that it is the cosmic-ray density that scales like the stellar birth rate, and not the number of γ -ray point sources. In the regions of normalisation used above, the contribution of cosmic ray interactions with interstellar matter is of the order of 1.3×10^{-5} photons

> 100 MeV $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ (ref. 14) if one takes account of the latitude range $|b^{\text{II}}| < 6^\circ$. The average contribution of point sources to the combined flux of $\sim 2.3 \times 10^{-5}$ is then 43%. Had the distribution in apparent brightness for O-stars been fitted to the value for cosmic ray produced γ rays alone, a value of 9×10^{-5} photons $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$ would have been predicted for the galactic centre region (somewhat high (Fig. 4)), and 1.6×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{rad}^{-1}$, if one had normalised to the total flux of 2.3×10^{-5} (much too high; see Fig. 4).

Thus one may scale only one of the two components, cosmic ray interactions or point sources, but not both. Therefore, one will have to argue either (as refs 14, 33, 35–37, for example) that the point source contribution remains at a comparatively low level throughout the galaxy, and the γ -ray emissivity through cosmic-ray matter interactions scales like the stellar birth rate, or one may hypothesise that the γ -ray point sources are correlated with the stellar birth rate which then requires that the cosmic ray produced γ -ray emissivity remains at a low and perhaps even constant level throughout the galaxy. If one considers the consequences of the second hypothesis, that it is the point sources which follow the stellar birth rate, then 7–8 γ -ray point sources would have been expected to lie within the Cos B viewing direction centred at 74° , while only three have yet been discovered¹⁷, and 14 should have been visible during the observation period centred at 322° , yet again only three have been seen¹⁷. 20 point sources would be expected from an observation period directed towards the galactic centre. As the longitude interval of any Cos B observation period is only $\sim 30^\circ$ wide one can clearly understand, why the γ -ray instrument cannot resolve these sources. Also, the large number of sources that are expected to lie within any viewing direction may account for the fact that the energy spectra as measured by Cos B do not change significantly as a function of galactic longitude².

Hermesen *et al.*¹⁷ argued that the individual γ -ray point source has a luminosity of $\geq 10^{35}$ ergs s^{-1} , then approximately 5,000 point sources in the entire galactic disk are required to produce the bulk of the 10^{42} photons s^{-1} above 100 MeV that the galaxy emits.

The considerations made above, of course, just demonstrate a possibility, albeit an interesting one. They are, first limited by statistics as there is a 40% error in six point sources. Second, it is a hypothesis that γ -ray sources are correlated to the stellar birth rate and thus to the population of O-stars, and that in addition such a correlation can be extrapolated from the outer regions $l^{\text{II}} = 180^\circ \pm 80^\circ$ to the entire galactic disk.

On the other hand, the suppositions made here provide a powerful stimulus for further observational and theoretical investigations if such a correlation can be substantiated, and what the physical reasons behind it are.

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articles

Analysis of ^{36}Cl in environmental water samples using an electrostatic accelerator

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A new atom identification and detection technique for ultrasensitive mass spectrometry has been applied to the radionuclide ^{36}Cl . Measurements of the $^{36}\text{Cl}/\text{Cl}$ ratio in AgCl precipitated from six different natural water samples fell in the range 1.2×10^{-13} – 2×10^{-12} with a background level below 3×10^{-15} . For each sample 70 mg of AgCl was used, requiring 1–5 l of water.

CHLORINE-36, the longest lived radioactive isotope of chlorine, is continuously being produced in the atmosphere. In contrast with atmospheric ^{14}C , which forms a large reservoir as gaseous CO_2 , ^{36}Cl leaves the atmosphere in rain and enters the hydrosphere directly. Due to the low rate of exchange with solids, the soluble chlorides containing ^{36}Cl form an effective tracer in groundwater hydrology, and it has been suggested that the 308,000 yr half life would make it a promising clock for a chronology that could cover the entire Pleistocene era¹. Recently, the advantages of using ^{36}Cl to study groundwater were stressed² in connection with establishing the suitability of underground sites for the storage of nuclear wastes. However, before a ^{36}Cl chronology can be established, the origin and production rates of ^{36}Cl must be determined accurately and the measured concentrations related to hydrogeological and palaeoclimatological data. Such detailed and difficult studies should be stimulated by a demonstration here that ^{36}Cl can be detected in small samples with sufficient sensitivity.

The few measurements of ^{36}Cl in environmental samples published to date have been made by counting β emissions from the radioactive decay. Such experiments are difficult because of the low counting rates resulting from the long half life of ^{36}Cl . In addition, it is difficult to distinguish the β rays of ^{36}Cl from those of trace quantities of other radionuclides in the sample because of their low energy³. Tens of grammes of chloride taken from thousands of litres of water are required to give counting rates distinguishable above the background and even then counting times as long as a week are required. This article describes the first successful application of the Rochester MP tandem van de Graaff accelerator system as an ultrasensitive mass spectrometer^{4–6} to the problem of determining the $^{36}\text{Cl}/\text{Cl}$ ratio in tens of milligramme chloride samples taken from 1 to 5 l of water from various sources. For the 45 mg background sample

of zone refined AgCl reagent a concentration of $< 3 \times 10^{-15}$ was determined, equivalent to an activity of about $1 \beta \text{ ray yr}^{-1}$.

To use ^{36}Cl for dating groundwater, the input of ^{36}Cl must be determined for a region and assumed to be relatively constant in the past. Atmospheric ^{36}Cl originates from interactions of cosmic rays and their secondaries with ^{40}Ar and ^{35}Cl , from nuclear explosions, and possibly from the influx of meteoric dust. At the surface of the Earth ^{36}Cl is produced through the activation of ^{35}Cl by cosmic ray secondary neutrons and in the subsurface by neutrons from the natural radioactivity in uranium and thorium deposits⁷. To use the $^{36}\text{Cl}/\text{Cl}$ ratio, the dilution from old chlorine must also be understood. Atmospheric chlorine arises primarily from ocean spray, while additional chlorides enter groundwater from dissolution of salt deposits. Measuring the $^{36}\text{Cl}/\text{H}_2\text{O}$ ratio instead of the $^{36}\text{Cl}/\text{Cl}$ ratio corrects for an unknown dilution by old chlorine, but uncertain precipitation and evaporation rates complicate the analysis. Although some progress has been made in predicting quantitatively the above origins of ^{36}Cl and Cl in water, it is beyond the scope of this paper to discuss them in detail.

The samples studied, listed in Table 1, were chosen partly to cover a wide range in expected ^{36}Cl content and partly for reasons of convenience. The Rillito River sample, taken from a well near an ephemeral stream, is largely runoff from recent rainwater and is therefore expected to contain a high ^{36}Cl content from nuclear explosion tests. In an attempt to exclude the bomb input source, a sample was obtained from a deep well near Tucson, Arizona that had been estimated⁸ by means of tritium and ^{14}C analyses to be more than 2,000 yr old. As dilution from old salt deposits was also expected to be low, the $^{36}\text{Cl}/\text{Cl}$ ratio in this sample should provide a direct indication of the cosmic ray production rate in the atmosphere—the most important result required for establishing a ^{36}Cl chronology. Also studied were two samples from the Lake Ontario water intake of Eastman Kodak, a reagent grade sample that presumably originated in a mine (and thus should contain little ^{36}Cl), and two samples artificially enriched in ^{36}Cl .

Separation and identification of interfering isotopes

The methods and results for atom identification and detection of ^{14}C in natural samples were discussed in detail at a recent conference on radiocarbon dating⁹, where the first detection of

^{36}Cl , using a tandem accelerator¹⁰, and an attempt to detect ^{36}Cl using a cyclotron¹¹ were also reported. The present apparatus (Fig. 1) incorporates some important improvements over that used for the ^{14}C measurements¹³ which were found to be necessary to resolve the more closely spaced isotopes at the higher mass. An advantage of accelerating ^{36}Cl to energies above 1 MeV AMU^{-1} is that the atomic number Z can be identified by measuring the rate of energy loss of the ion. This Z determination, combined with the usual selection of a specific mass-to-charge ratio M/q with a combination of magnetic and electrostatic analysers, allows complete and unambiguous isotope identification without the severe loss in sensitivity typical of conventional high-resolution mass spectrometers. Although this analysis is adequate for positive identification of ^{36}Cl , in practice, the counting rate in the detector arising from trace impurities in the sample was high enough to produce gain shifts in the detector and produce tails that overlap the ^{36}Cl peak.

The basic limitation in measuring small concentrations of ^{36}Cl with mass spectrometers is due to the necessity of separating ^{36}Cl from the isobars ^{36}Ar and ^{36}S and from mass 36 molecules. Although chlorine readily forms stable negative ions, which is a requirement for acceleration in a tandem van de Graaff, argon (a noble gas) does not. No evidence was seen for argon in the detector (<1 argon atom per 10^{14} chlorine atoms) indicating that either Ar^- is not produced in the caesium sputter source or that it does not have a lifetime sufficiently long to reach the tandem terminal as a negative ion. Sulphur, however, forms negative ions almost as readily as chlorine, so, with a ^{36}S abundance of only 0.014%, a one p.p.m. sulphur impurity produces an interference at the 10^{-10} level. The great sensitivity of the present technique is illustrated by the fact that a 1 p.p.m. concentration, considered very low for a sulphur impurity, would still result in a ^{36}S count rate of 300 c.p.s. for typical conditions in this experiment. The range separation technique¹⁴ is not applicable because sulphur has the longer range so that special sample preparation techniques¹⁵ were necessary to reduce the sulphur content to <10 p.p.m. In addition, the heavy-ion counter¹² was modified to give a ^{36}S rejection rate of better than 10^6 to 1 (see Fig. 2).

Although interference from mass-36 molecules was eliminated by choosing a high charge state from the post-acceleration analysis system, atomic fragments that result from breakup of the mass-36 molecules at the terminal are not separable from ^{36}Cl in the magnetic and electrostatic analysers if they happen to have the same value of M/q after acceleration as the ^{36}Cl ions. For example, the $^{12}\text{C}_3^-$ molecular ion, known to be produced in nA quantities¹⁸ by sputter sources, will behave exactly as $^{36}\text{Cl}^{6+}$ in electric and magnetic fields when stripped to $^{12}\text{C}^{2+}$ in the terminal. Although these carbon ions could be distinguished by their energy loss in the detector, the counting rate would be

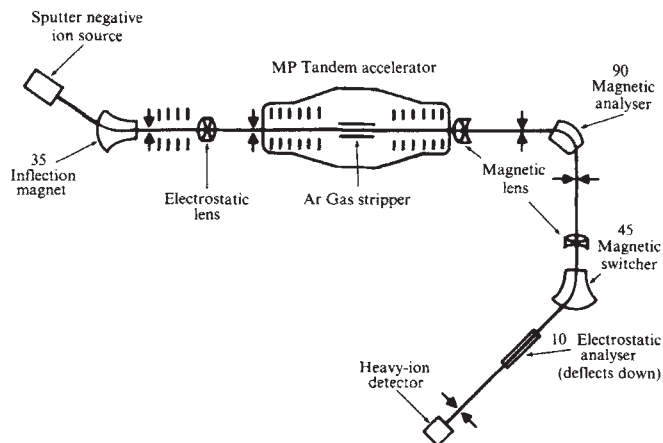


Fig. 1 Diagram of the apparatus used for ^{36}Cl measurements. From 5 to $13\text{ }\mu\text{A}$ of Cl^- ions were produced from AgCl or NaCl samples in a caesium sputter source (model 834 HICONEX, General Ionex Corp.) the mass-36 negative ions were analysed by a magnetic inflection system ($M/\Delta M = 25$), accelerated to 170 KeV, then injected into the Rochester Mp tandem accelerator. At the terminal, which was held at 10.00 MV by a generating voltmeter stabilisation system, the ions were stripped of several electrons by argon gas, then accelerated again to ground potential. Charge state 7, mass 36 ions with $E = 80.17\text{ MeV}$ were selected by a 90° magnet ($P/\Delta P = 600$), bent by a 45° magnet (which served to eliminate scattered particles), deflected 10° out of the horizontal plane by electrostatic plates ($E/\Delta E = 200$), and then analysed by a heavy-ion ionisation counter¹². By increasing the inflection magnet and analysing magnet fields the ^{37}Cl beam could be switched onto the Faraday cup following the analysing magnet—the beam current measured here was used for normalisation.

excessive even for a very low level of carbon impurity. This type of background can be eliminated by choosing a charge state that has no common factor with 36, that is 5, 7, 11, 13 or 17. For 10 MeV chlorine in argon gas, the charge-state distribution peaks near 6 with a full width at half-maximum of about 3 charge units¹⁸, thus leaving 5 and 7 as the only good choices. Charge-state 7 was chosen for its higher energy (80 MeV as opposed to 60 MeV for charge-state 5) allowing better separation from ^{36}S in the detector.

Other potential sources of interference are $^{35,37}\text{Cl}$ ions which are injected into the tandem from tails of the intense $^{35,37}\text{Cl}^-$ beams at the ion source because of the limited resolution of the inflection magnet (^{35}Cl can also be injected directly as H^{35}Cl^-). Although most of these ions will leave the tandem at discrete energies that are not selected by the 90° analysing magnet, some will charge exchange a second time in the residual gas of the

Table 1 Results for ^{36}Cl measurements

Sample	Total run time (min)	Total ^{36}Cl counts	Number of runs	Concentration $^{36}\text{Cl}/\text{Cl} (\times 10^{15})$	Activity (d.p.m. - kg Cl)	Cl/ H_2O ($\text{mg}^{-1}\text{ l}$)	$^{36}\text{Cl}/\text{H}_2\text{O}$ ($\times 10^6$) (atoms $^{-1}$ l)
Groundwater, Rillito River (ephemeral), AZ	90	7,681	7	$1,950 \pm 150$	145	48	1,600
Surface water, San Pedro River, Tombstone, AZ	204	8,916	13	650 ± 110	48	4-12	~90
Groundwater, flowing well in St David, AZ	68	2,403	7	400 ± 40	30	8	53
Subsurface water, Lake Ontario, NY (May, 1978)	41	2,062	4	210 ± 20	15	27	95
Subsurface water, Lake Ontario, NY (June, 1978)	172	4,555	9	120 ± 20	9	27	54
AgCl reagent, zone refined	57	32	3	3 ± 1	0.2	—	—
NaCl enriched to $^{36}\text{Cl}/\text{Cl} \approx 33,000 \times 10^{-15}$	13	21,799	2	$18,500 \pm 900$	1,320	—	—
NaCl above diluted to $^{36}\text{Cl}/\text{Cl} \approx 330 \times 10^{-15}$	58	975	4	250 ± 30	18	—	—

Although a few hundred mg of AgCl was obtained from the natural water samples, only 16-66 mg was used in the ion source, and most of this remained after the experiment. Errors quoted are one standard deviation for several measurements. Specific activity is that expected based on the ^{36}Cl concentration measured. The approximate total chlorine concentration in the original water sample is given in the next to last column, followed by the ^{36}Cl concentration.

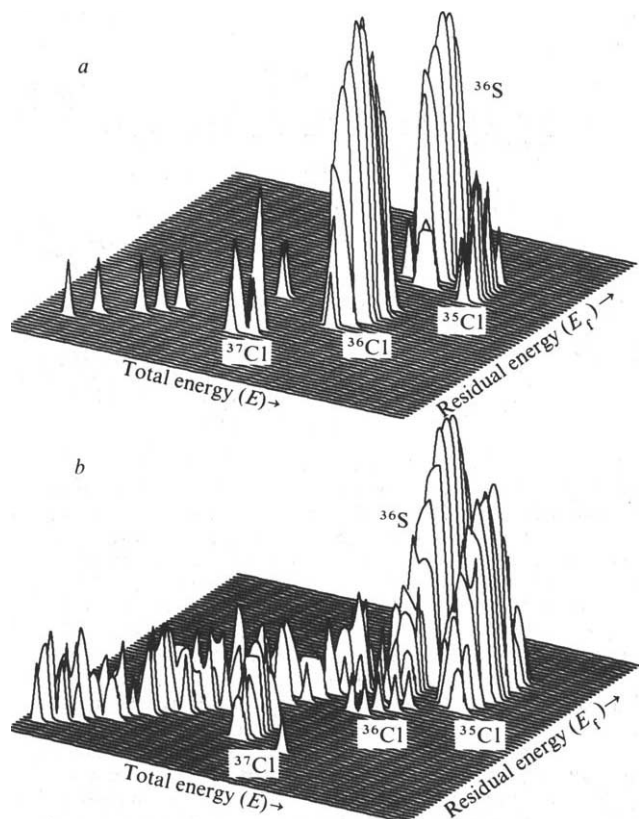


Fig. 2 Two-parameter logarithmic spectra for single runs on: *a*, a sample from Lake Ontario; and *b*, zone refined reagent grade AgCl. To help remove the effect of a high-energy tail arising from the Landau-Symon effect¹⁶ on the intense ^{36}S peak in the energy loss spectrum (not shown), the heavy-ion counter¹² was modified to include four energy loss (ΔE) plates and a smaller residual energy (E_t) plate. In the computer, a digital window was placed on each of the first three ΔE signals at the expected location of ^{36}Cl . This reduced the effect of the tails since the high energy-loss events that produce them are not correlated among the different ΔE measurements. The events passing the three ΔE gates and an additional hardware E_t gate were plotted on the two parameter display of E_t against E shown. The long ^{36}S tail results from lower energy particles scattered into the counter. The zero has been offset on each axis.

acceleration tubes to produce $^{35,37}\text{Cl}$ ions with the same momentum and charge state as the ^{36}Cl ions. With only the ME/q^2 selection of magnetic analysers (all that is normally used with accelerators) the $^{35,37}\text{Cl}$ count rate was about 10^5 c.p.s. The intensity of this continuum was reduced more than 5 orders of magnitude with the E/q selection of an electrostatic analyser installed for these ^{36}Cl measurements (see Fig. 1). The remaining $^{35,37}\text{Cl}$ ions were easily resolved from ^{36}Cl in the detector.

Results and discussion

The results are given in Table 1. Normalisation for the $^{36}\text{Cl}/\text{Cl}$ ratio was obtained by reading $^{37}\text{Cl}^{7+}$ beam current before and after each ^{36}Cl measurement. As this current could not be monitored continuously, the accuracy of a measurement was limited by any fluctuations in the source output and accelerator transmission efficiency. The standard deviation of the $^{36}\text{Cl}/\text{Cl}$ ratios from several 10–20-min runs provided a measure of this uncertainty and was usually 10–15%, which is 2–4 times larger than the statistical error for single runs. In subsequent ^{14}C experiments the cycle period was decreased to 3 min, and this uncertainty, when calculated for the mean of several cycles, was reduced to about 3%. There is no reason that this level of

uncertainty could not now be obtained for ^{36}Cl , where 3% corresponds to an age uncertainty of 13,000 yr.

The experimental method was checked by using two samples enriched in ^{36}Cl : the first was made by exposing reagent grade NaCl (BDH, lot no. 81515–7203) to an approximately known neutron flux from the University of Toronto Slowpoke reactor, the second by dilution of the first. The measured ratio of ^{36}Cl content, 74 ± 10 , is in marginal agreement with the expected value of 100. This discrepancy would be resolved if the original reagent contained ^{36}Cl with a $^{36}\text{Cl}/\text{Cl}$ ratio as low as 30×10^{-15} (the ^{36}Cl content of the reagent used was not measured and was assumed to be negligible when determining the dilution factor of 100). A comparison between the absolute $^{36}\text{Cl}/\text{Cl}$ ratios is not useful because of the not-so-well known neutron flux. Clearly, more calibration checks need to be made before detailed hydrology studies are undertaken.

The low result for the zone-refined reagent-grade AgCl (Fisher catalog no. S174, lot no. 742272), demonstrating a sensitivity of three parts in 10^{15} , is probably the most significant result of this work. The sensitivity may be greater than this value as it could not be determined what fraction of the 32 counts collected was attributable to the ^{36}S tail (see Fig. 2) or how much was due to cross-contamination from other samples in the ion source. Even when considered as an upper limit to the sensitivity, the result shows that this technique is adequate for dating samples with ages up to 6 half lives or 2×10^6 yr. Memory effects and cross-contamination were shown to be small because the non-diluted enriched sample was run for ~4 h before experiments on the refined sample and so a dynamic range of almost 10^4 was realised.

Interpretation of the results for the neutral samples is difficult due to the present lack of detailed hydrogeological data, but H. Bentley helped us draw the following preliminary conclusions. Although the Rillito River sample was taken from groundwater, it is expected to be quite young—the higher ^{36}Cl content probably reflects input from nuclear testing in the atmosphere. Irrigation of nearby fields is indicated as the source of the high chloride content of the sample, and as this additional chlorine is atmospheric in origin, it may contain appreciable ^{36}Cl . The San Pedro River sample, on the other hand, is from surface water that is fed by groundwater springs—the relatively low concentration of ^{36}Cl may indicate that the bulk of this water is pre-bomb in origin. In contrast, the St David and Tucson well samples are certainly pre-bomb in origin⁸—these results should indicate the base atmospheric cosmic ray production for water that has not aged long compared to the ^{36}Cl half life. The Lake Ontario samples probably give a low $^{36}\text{Cl}/\text{Cl}$ result because of dilution from the great quantities of de-icing salt¹⁹ used near the Great Lakes in the last decade. The $^{36}\text{Cl}/\text{H}_2\text{O}$ ratio, not affected by the de-icing salt, is small compared to the Rillito river value because of the higher precipitation and lower evaporation rates in the Great Lakes region as compared to Arizona.

In conclusion, the method described can be used to measure the ^{36}Cl concentration in a 100-mg sample of AgCl with an uncertainty of <10% in less than 1 h. This performance should be adequate for studies using ^{36}Cl as a tracer in groundwater hydrology. Among other geophysical dating applications likely to benefit from the new technique is the study of deep ice core samples in Greenland and Antarctica²⁰. Also, important information about the cosmic ray containment time within the Galaxy can be inferred from the number of the long-lived radionuclides ^{10}Be , ^{26}Al , ^{36}Cl , and ^{129}I present in the cosmic ray flux²¹. The concentration of these isotopes in Moon rocks²² or meteorites²³ might be indicative of that flux, and a depth profile may give information on the cosmic ray energies.

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Sm–Nd systematics of Lewisian gneisses: implications for the origin of granulites

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Sm–Nd isotope systematics of Lewisian gneisses from North-west Scotland suggest that their igneous calc-alkaline precursors separated from previously undifferentiated mantle 2,920±50 Myr ago. Approximately 200 Myr elapsed before this material was finally differentiated and stabilised as granulite and amphibolite facies crust.

DURING the past decade, measurements of Sr-, Nd- and Pb-isotope ratios have demonstrated that ancient crustal segments are, in general, stabilised shortly after the separation of their igneous precursors from the mantle. For example, the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the gneisses comprising many Archaean crustal segments are found to be close to the estimated mantle $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for the time of their stabilisation, an observation which precludes more than ~100–200 Myr (refs 1–5) residence in a high Rb–Sr crustal environment. These conclusions, which are generally accepted amongst isotope geochemists, sometimes seem contrary to the geological complexity of such regions, as deduced from field observations and from the substantial geochemical changes which the igneous precursors must have experienced before they became structurally and thermally stable continental crust. Notable amongst these latter changes is the marked depletion of heat-producing elements (K, U and Th)^{5–11} and some other lithophile trace elements^{12–16} in granulite facies rocks.

The time interval between the initial generation of sialic material from the mantle and the eventual stabilisation of continental crust, during which these latter geochemical changes occurred, can only be broadly constrained by Rb–Sr and U–Pb techniques as indicated above. However, the successful application of the ^{147}Sm – ^{143}Nd system to the dating of Archaean rocks^{17–19} indicates that a combination of Sm–Nd, U–Pb and Rb–Sr studies may produce the time resolution required. The considerable amount of Rb–Sr (refs 4, 20–24) and U–Pb (refs 4, 10, 11, 24–26) geochronological data already existing for the Archaean Lewisian gneisses of North-west Scotland makes this a favourable location for an attempt to resolve this time interval.

The Lewisian gneiss complex forms the western foreland to the Caledonian orogenic belt, and crops out on the mainland of North-west Scotland, on the Outer Hebrides and several of the islands of the Inner Hebrides, and as large deformed slices

within the Caledonian belt itself. Detailed geological field studies beginning with Sutton and Watson²⁷, geochemical studies^{15,30}, and numerous geochronological investigations using K–Ar (refs 24, 28, 29), Rb–Sr (refs 4, 20–24) and U–Pb (refs 4, 10, 11, 24–26) techniques have served to subdivide the Lewisian into three main regions. The central region contains pyroxene gneisses which have crystallised at high temperatures and pressures^{31–33}, and which record structural and chronological evidence for the earliest (Scourian) granulite facies metamorphism ~2,700 Myr ago. The northern and southern regions are composed mainly of amphibolite facies gneisses whose relationships with the central region granulites have been considerably discussed, but are now widely regarded as having occupied an initially higher structural level in the crust than the granulites³⁰. The amphibolite facies regions record evidence of two later periods of deformation, metamorphism and igneous activity at ~2,200 Myr (Inverian) and ~1,700 Myr (Laxfordian). These metamorphic events have locally affected the central region granulites causing retrogression to amphibolite facies mineral assemblages^{30,34}. The samples used for this study came from the central region, near the village of Drumbeg, Assynt, and from the northern region, near Rhiconich, and have been described elsewhere^{15,30,35}.

Of the large amount of geochronological data which now exists for various components of the Lewisian complex, the more pertinent recent data of reasonable precision are summarised in Table 1. The three zircon suites analysed by Pidgeon *et al.*^{25,26} yield U–Pb concordia–discordia intercept ages that are almost within error at 2,700 Myr. This age is interpreted as dating zircon growth during Scourian metamorphism. These data define lower intercept ages corresponding to well-defined geological events, namely the Laxfordian and the Caledonian. Zircon data from Lewisian gneiss in the northern region, near Durness²⁴ are enigmatic, giving an older concordia–discordia age of 2,810±50 Myr and a lower intercept age of 1,040±50 Myr.

The Pb–Pb and Rb–Sr whole rock ages for Lewisian gneisses in Table 1, though of poorer precision, agree with the Scourian ages obtained from zircon studies.

Sm–Nd systematics

The Sm and Nd contents and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios (Table 2) have been measured on 11 samples of Lewisian orthogneiss including

Table 1 Lewisian geochronologic data

Age* (10 ³ Myr)	Samples	Locality	Method	Ref.
2.63 ± 0.14	Whole-rock amphibolite facies gneisses	Southern Outer Hebrides	Rb-Sr	4
2.60 ± 0.12	Whole-rock amphibolite facies gneisses	Southern Outer Hebrides	Pb-Pb	4
2.86 ± 0.20	Whole-rock amphibolite and granulite facies gneisses	Outer Hebrides and N, S and central regions	Pb-Pb	10
2.68 ± 0.06	Whole-rock granulite facies gneisses	Scourie	Pb-Pb	11
2.81 ± 0.05	Zircon size fractions from quartzofeldspathic gneiss	Rispond, Durness, northern region	Concordia-discordia intercept	24
2.73 ± 0.01	Zircon size fractions from quartzofeldspathic gneiss	Harris	Concordia-discordia intercept	26
2.66 ± 0.02	Two zircon suites from granulite facies gneisses	Kylesku, central region	Identical concordia-discordia intercepts	25

* Errors are quoted at the 2 σ level. Ages here and in the text conform to the following decay constants:

$$\lambda^{235}\text{U} = 9.85 \times 10^{-10} \text{ yr}^{-1}; \lambda^{238}\text{U} = 1.551 \times 10^{-10} \text{ yr}^{-1}; \lambda^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}; \lambda^{147}\text{Sm} = 6.54 \times 10^{-12} \text{ yr}^{-1}$$

both granulites and amphibolites. Previous major and trace element analyses of the Lewisian indicate an igneous origin from a plutonic suite with calc-alkaline affinities^{14,30,35-37}. Analytical procedures for the chemical separation and subsequent mass-spectrometric analysis of Sm and Nd have been described elsewhere^{17,38}. The Sm/Nd ratios are mostly less than the bulk earth value (0.31) (refs 17-19, 39, 40), corresponding to light rare-earth enriched patterns; however, two basic granulites are slightly light rare-earth depleted and have Sm/Nd ratios up to 0.372.

The data are plotted on a Sm-Nd evolution diagram in Fig. 1. The best fit line⁴¹ has a slope corresponding to an age of $2,920 \pm 50$ (2 σ) Myr ($\lambda^{147} = 6.54 \times 10^{-12} \text{ yr}^{-1}$) and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.508959 ± 49 (2 σ). All the gneiss samples, irrespective of locality or metamorphic grade, conform closely to the best-fit line indicating that their igneous parent materials were derived contemporaneously and from source materials of similar history.

The Sm-Nd age obtained is significantly older than reliable ages obtained by whole-rock Rb-Sr and Pb-Pb and zircon U-Pb methods (see Table 1). The Pb-Pb and Rb-Sr data on whole rocks are consistent with a short time interval between formation of the igneous precursors and their chemical differentiation. The Sm-Nd age demonstrates that this is close to 200 Myr and the age is thus interpreted as the time of mantle-crust differentiation to produce the calc-alkaline precursors to the Lewisian. Specifically the time interval between the Pb-Pb and Sm-Nd whole-rock ages, $\Delta T_{\text{Nd-Pb}}$, is equal to 240 ± 90 Myr (uncertainties in the decay constants used should not significantly change this value).

Present day oceanic basalts exhibit a wide range of Nd compositions^{38-40,42-45} with $^{143}\text{Nd}/^{144}\text{Nd}$ ratios between 0.51251 and 0.51329. This variation implies the existence of long lived mantle heterogeneities in Sm-Nd and an overall light rare-earth depletion ($\text{Sm}/\text{Nd} > \text{chondritic}$ ⁴⁶) of oceanic upper mantle. The volume of mantle differentiated to produce the continental crust, the rate of crustal growth and the magnitude of the concomitant preferential light rare-earth ($\text{Sm}/\text{Nd} < \text{chondritic}$) enrichment into the crust are critical aspects of the history of present-day mantle heterogeneity. The precise timing of crust-mantle differentiation events and the evolution of radiogenic isotopes before such events would provide powerful constraints on solutions to these problems. We have shown that mantle-crust differentiation may be precisely dated by the Sm-Nd method. Furthermore, inferences concerning the previous Sm-Nd environment may be made from the corresponding initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios.

From Sm/Nd and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios determined on Archaean single whole-rocks of known age, DePaolo and Wasserburg^{39,40} suggested that Archaean continental crust had grown by differentiation of mantle sources that had previously evolved with an approximately chondritic Sm/Nd ratio. On an age against initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio plot (Fig. 2) their data plot close to a model growth line defined by the chondritic Sm/Nd ratio of 0.31 (ref. 46) and a $^{143}\text{Nd}/^{144}\text{Nd}$ ratio (0.50682) (ref. 47) equal to that in the Angra dos Reis achondrite at 4,550 Myr ago. Their contention has been strongly supported by the Sm-Nd whole-rock isochron studies of Hamilton *et al.*¹⁷⁻¹⁹ on Archaean igneous rock suites. The ages and initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios obtained by the latter also plot close to the chondritic growth

Table 2 Sm-Nd* data on Lewisian gneisses from the granulite facies zone (Drumbeg, Assynt) and the northern amphibolite facies zone (Rhiconich)

Rock type, locality (grid. ref.)	Sample	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd} \pm 2\sigma$
Basic gn., Drumbeg (NC128327)	7D	1.749	5.366	0.1960	0.512756 ± 24
Gt-basic gn., Drumbeg (NC107332)	8X	2.138	5.480	0.2346	0.513518 ± 30
Basic gn., Drumbeg (NC113340)	7N	2.289	8.064	0.1707	0.512195 ± 30
Tonalitic gn., Drumbeg (NC099325)	12F	2.146	11.92	0.1082	0.511006 ± 34
Tonalitic gn., Drumbeg (NC136312)	14F	10.33	83.81	0.0741	0.510376 ± 20
Tonalitic gn., Drumbeg (NC165319)	15M	2.554	17.54	0.0875	0.510634 ± 32
Tonalitic gn., Drumbeg (NC094328)	18T	2.650	15.46	0.1030	0.510962 ± 28
Tonalitic gn., Drumbeg (NC193336)	19X	3.062	23.75	0.0775	0.510501 ± 24
Granodioritic gn., Rhiconich (NC280510)	5218	7.694	41.05	0.1127	0.511143 ± 26
Granodioritic gn., Rhiconich (NC260530)	5326	5.776	39.87	0.0871	0.510638 ± 30
Tonalitic gn., Rhiconich (NC220540)	5422	5.631	33.88	0.0999	0.510902 ± 36

* All ratios atomic. † gn., gneiss.

line (Fig. 2), with Sm/Nd ratios computed for the time intervals from mantle-crust separations to 4,550 Myr being within error of 0.31. The time integrated Sm/Nd ratio for the igneous precursors to the Lewisian is 0.311 ± 4 .

Several studies⁴⁸⁻⁵⁰, including the present one, indicate that crustal metamorphism does not result in significant fractionation of Sm and Nd or perturbation of Sm-Nd isotope systematics. The major fractionation of Sm and Nd during Earth evolution is thus through the initial differentiation of the crust from the mantle. Using this fact and assuming differentiation of Archaean crustal segments from a chondritic Sm-Nd mantle environment, McCulloch and Wasserburg⁵⁰ computed model Sm-Nd ages from Sm/Nd and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios measured on composite

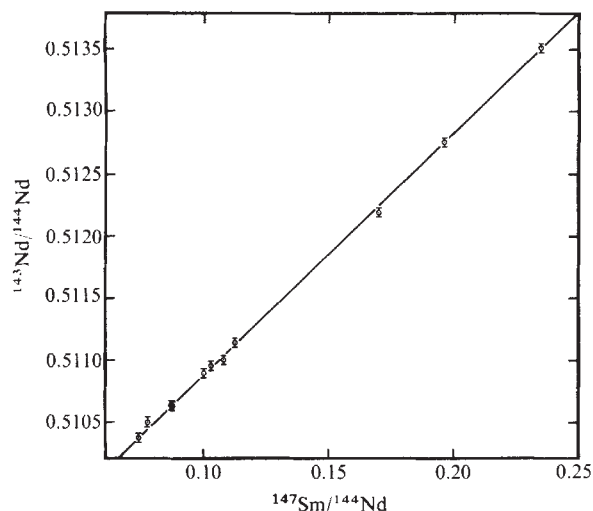


Fig. 1 Sm-Nd evolution diagram for the Lewisian gneisses. Age = $2,920 \pm 50$ Myr; initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio = 0.508959 ± 49 .

Canadian Shield samples. The model ages of 2,700–2,500 Myr agree with the available geological ages and give evidence of the derivation, within a short time interval, of a major crustal segment from essentially chondritic Sm-Nd reservoirs.

Differentiation of continental crust and granulite formation

Whole-rock Pb-Pb data^{4,10,11} for Lewisian granulites indicate that depletion of U (and by inference of Th) and alkali elements (K, Rb, Cs), continued to 2,700 Myr some 200 Myr after separation of the parent material from the mantle. The low concentrations of K, Rb, Cs, Th and U in Lewisian granulites (and possibly many other Precambrian granulites⁵¹) are not primary features of their igneous precursors, but are the result of metamorphic purging of these elements from the lower crust during a time interval of ~200 Myr. The characteristic depletion of alkali elements in granulites results in low Rb/Sr ratios, often lower than the bulk earth value (0.03) (refs 32, 33). This is particularly true for the Lewisian samples which have Rb/Sr ratios averaging about 0.02 (ref. 14). In contrast, amphibolite facies components of the Lewisian have Rb/Sr ratios higher than the bulk earth value. For example, the amphibolite facies gneisses studied by Moorbath *et al.*⁴ have an average Rb/Sr of 0.4. The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7014 at 2,670 Myr obtained for these samples requires a time-integrated Rb/Sr ratio in the interval 2,920–2,670 Myr which is about an order of magnitude lower at ~0.03 but is still higher than that for the granulitic gneisses. Thus the amphibolitic gneisses may have acquired their high Rb/Sr ratios as a result of metamorphism and crustal differentiation. It seems feasible that some of the alkali elements lost from the granulite facies rocks during metamorphism migrated to higher structural levels.

Comparative rare-earth element (REE) abundance studies of granulite and amphibolite facies rocks^{48,49} indicate that REE are

not substantially fractionated by even high grade metamorphism and the data included here are in accord with these observations (see Table 2). Furthermore, the strong light REE enrichment seen in Lewisian granulites of acid to intermediate composition is compatible with their calc-alkaline nature and is not readily reconcilable with the theory that the heat producing and some other lithophile trace elements were removed in a partial melt as suggested by Fyfe⁵². Not only would such a mechanism tend to leave a residue with less fractionated rare-earth patterns than observed for most Lewisian granulite facies gneisses, but it would also tend to eradicate the isotopic evidence for earlier events. Crustal differentiation through upwards migrating non-magmatic fluids as proposed in more specific ways by Heier⁶ and by Tarney and others⁵³ is supported by this study. On the other hand, the gross extension of such models to remove heavy rare earth and other elements from the Archaean lower crust by hydrothermal fluid transport, as recently proposed by Collerson and Fryer⁵⁴, is not supported by the Nd isotope data.

Is the time interval, $\Delta T_{\text{Nd-Pb}} = 240 \pm 90$ Myr, (during which crustal differentiation and stabilisation of granulites occurred), compatible with simple thermal considerations? To determine this we have assumed that within the interval $\Delta T_{\text{Nd-Pb}}$ the Lewisian crust can be modelled as a semi-infinite solid, containing 1 wt % K, (a conservative estimate), and $K/U = 10^4$ and $\text{Th}/U = 4$ by weight. Phase equilibrium considerations³¹⁻³³ indicate that at the end of this time interval the granulites were at a depth of at least 35 km, corresponding to a pressure of $\sim 10^4 \text{ Nm}^{-2}$ (1 GPa) and a temperature of ~1,100 K. If the temperature distribution in the Lewisian 2,920 Myr ago is conservatively estimated as increasing linearly with depth to 570 K at 35 km and it is further assumed that the heat input from the mantle is zero, then the subsequent temperature distribution as a function of time can be readily computed⁵⁵. For values of the conductivity and thermal diffusivity of $3.3 \text{ W m}^{-1} \text{ K}^{-1}$ and $1.6 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$, respectively⁵⁶, it is found that the temperature at 35 km depth increases to 1,150 K after 50 Myr (2,870 Myr ago) and to 1,570 K after 100 Myr (2,820 Myr ago). This result indicates that extensive melting of the lower crust, even under anhydrous conditions, would occur by 2,820 Myr, unless the heat-producing elements migrated to higher structural levels

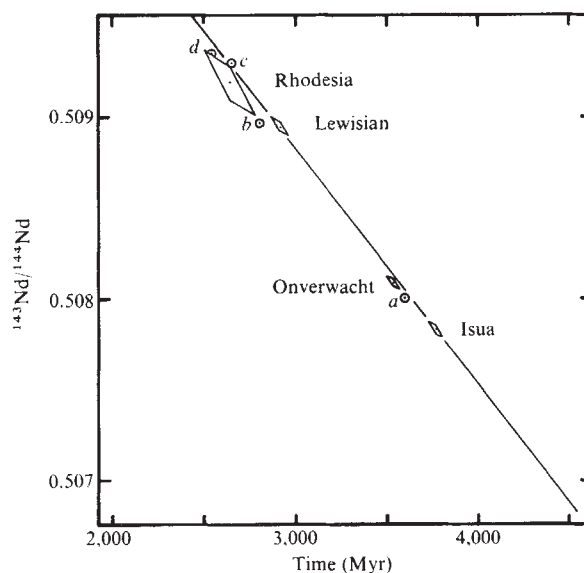


Fig. 2 Initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios of Archaean rocks plotted against age determined from whole-rock isochron data. Line represents growth of $^{143}\text{Nd}/^{144}\text{Nd}$ in a chondritic reservoir which had a $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50682 at 4,550 Myr ago. Data from DePaolo and Wasserburg^{39,40}, Hamilton *et al.*¹⁷⁻¹⁹ and this paper. a, Amitsoq gneiss, Western Greenland; b, Preissac-Lacorne granodiorite, Superior Province, Canada; c, Fiskenaeset anorthosite, Western Greenland; d, Great Dyke, Rhodesia.

before this time. This conclusion holds for reasonable values of conductivity and thermal diffusivity and it should be emphasised that our estimates of K content and initial temperature distribution are conservative and that the mantle heat input was equated to zero. From these simple thermal considerations we may conclude that to avoid extensive melting, heat producing elements must have commenced migration from the lower crust <100 Myr after crustal thickening. The mechanism of this migration is still not clear but did not involve a transport medium which would significantly affect the Sm-Nd systematics, as would a magmatic phase.

The combined application of Sm-Nd, U-Pb and Rb-Sr systematics to ancient crustal terrains can provide important constraints on the early phases of crustal differentiation and stabilisation. This is a simple reflection of the markedly different mobilities of these elements during crustal differentiation processes. At least in the case of the Lewisian crust, the completion of metamorphic differentiation and granulite formation succeeded the primary igneous differentiation of parent materials from the mantle by 240 ± 90 Myr. The heat producing elements were transported to higher crustal levels from where heat could be readily dissipated, and the granulite facies lower crust was maintained in a more refractory and thermally stable condition.

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Pc 1 Duarte, a common polymorphism of a human brain protein, and its relationship to depressive disease and multiple sclerosis

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Pc 1 Duarte, a common variant of a human brain-specific protein, is present in one-third of the control population and has a gene frequency of 0.17. There was a significantly increased frequency of mutant heterozygotes and homozygotes among individuals with depressive disease. This is consistent with a model in which Pc 1 Duarte is the major gene in depressive disease, acting in conjunction with an environmental threshold effect.

THE genetic basis of the major psychoses is poorly understood. In schizophrenia, twin, family and adoption studies clearly indicate the importance of genetic factors¹⁻³. The affective psychoses have been less extensively studied, but again family and twin studies provide strong evidence of genetic factors³⁻⁵. Proposed mechanisms for these psychoses include a single

autosomal dominant gene with reduced penetrance⁶⁻¹⁰, a single recessive gene¹¹, a predominantly recessive gene with an environmental threshold effect¹², polygenic inheritance^{5,13-15} and in the case of manic-depressive psychosis, X-linked inheritance¹⁶.

Several years ago I began a search for the mutant protein in Huntington disease using two-dimensional gel electrophoresis of many different extracts of brain and other tissues. Although the mutant protein in this disorder has not yet been found, a perchloric acid (PCA) extract of the caudate and putamen showed a commonly occurring mutant protein, which I call Pc 1 Duarte (ref. 17). By electrophoretic studies, it is absent from non-neural tissues and thus represents the first polymorphism of a human protein specific to nerve tissue. Because this protein is prominent in the caudate and putamen, the major site of dopaminergic neurones believed to be involved in schizophrenia^{18,19}, and because some genetic theories suggest a primarily

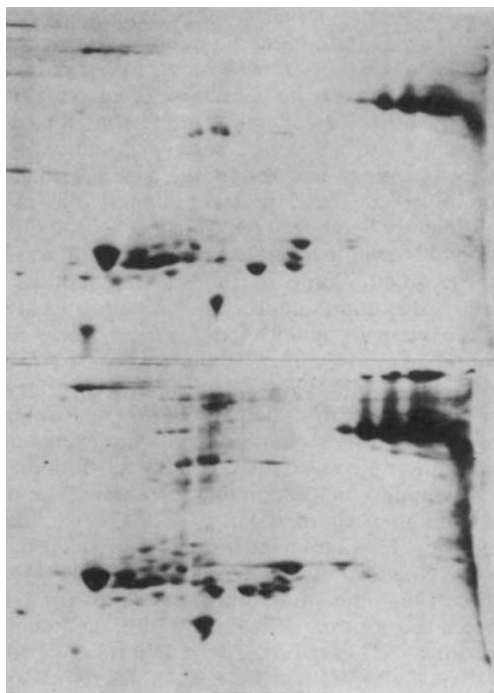


Fig. 1 Two-dimensional gel electrophoresis of PCA extracts of the caudate and putamen of homozygous normal (*Pc 1 A/Pc 1 A*) (top) and heterozygous (*Pc 1 Duarte/Pc 1 A*) (bottom) individuals. Frozen brain material was allowed to thaw and each gramme of tissue mixed with 3 ml of 0.13 M PCA plus 0.03 ml of phenylmethylsulphonyl fluoride (16 mg per ml ethanol). This was homogenised in a Teckmar homogeniser (Cincinnati) for 30–60 s. The homogenate was placed on a rocker at 4°C for 1 h then centrifuged 10,000 g for 20 min. The supernatant was dialysed against 0.05% β -mercaptoethanol for 4 changes then lyophilised. Electrophoresis was by the technique of O'Farrell⁴⁴ with the following modifications. Isofocusing was done in 3.7-mm i.d. Pyrex tubes with 12-cm long 5% acrylamide gel made from a stock solution of 25.5% acrylamide, 4.5% *N,N'*-diallyltartardiamide. The final gel contained 9.5 M urea, 2% NP40 and 2% BioRad ampholytes (2 parts 3–10, 6 parts 4–6, 4 parts 5–7, 1 part 7–9). The anode solution was 0.01 M phosphoric acid, the cathode solution 0.04 M NaOH, 0.02 M $\text{Ca}(\text{OH})_2$. The gels were electrophoresed at 500 V overnight. The pH gradient was read directly on the gel with a flat bed electrode. The second dimension was a 12.5% acrylamide–sodium bicarbonate gel as described by O'Farrell⁴⁴. After electrophoresis the gels were stained overnight in 0.05% Coomassie brilliant blue R dissolved in 10% acetic acid–25% isopropanol, destained for 2 h in 10% acetic acid–25% isopropanol, and destained an additional 24 h in 10% acetic acid. The gels were photographed with contrast process Ortho Kodak film with a yellow filter. To determine the molecular weight of *Pc 1 Duarte*, standards were included with sample in one gel and this compared with a gel without standards and with a gel of standards alone.

recessive but common mutant gene, it was of interest to determine if *Pc 1 Duarte* was associated with any of the major psychoses. Significant correlations were found with the depressive diseases and with multiple sclerosis.

Electrophoresis

Two-dimensional gel electrophoresis of the PCA extract of the caudate and putamen of a normal *Pc 1 A/Pc 1 A* (AA) individual and a *Pc 1 Duarte/Pc 1 A* (AD) heterozygote is shown in Fig. 1. The critical portion of the gel in these two individuals plus a *Pc 1 Duarte* homozygote (DD) are shown in Fig. 2 and the terminology is illustrated in Fig. 3. In the normal homozygote there is a major polypeptide, *Pc 1 AI*, and two minor satellite polypeptides, *Pc 1 AII* and *AI*. In the variant the major polypeptide is exactly split to form an additional, more basic protein, *Pc 1 Duarte I*. The two minor satellite polypeptides are also split to form *Pc 1 Duarte II* and *III*. Faint polypeptides *Ia* and *IIa* migrate just ahead of both the normal and variant *I* and *II*. The splitting of three polypeptides into six in the presence of the variant indicates that *AI*, *II* and *III* all have the same

polypeptide sequence and the separation into three forms is due to post-transcriptional modifications.

A single amino acid substitution leading to more basic mutant polypeptides explains the observed pattern. There was no correlation between this mutant pattern and drug intake, age, time from death to autopsy, or source of the brains. The patterns are highly reproducible. Many samples have been run two to three times, some seven times, with the same result.

There were approximately 20 major and 40 minor polypeptides in the PCA extracts. None of the other polypeptides were polymorphic. The *Pc 1* terminology (Perchloric acid extract) allows naming of *Pc 2*, *Pc 3* and so on, as other polypeptides are studied. The protein was not present in PCA extracts of fibroblasts, liver, kidney, spleen or lung and thus seems to be nerve tissue specific.

To examine the intracellular distribution of *Pc 1 A*, PCA extracts of various brain fractions were electrophoresed. *Pc 1 A* was present only in the 100,000g supernatant fraction, indicating it to be a cytosol protein and not intimately attached to the nucleus, myelin, synaptosomes, mitochondria or cell membranes.

To examine the distribution of this protein, PCA extracts of many parts of the brain were electrophoresed. *Pc 1 A* was most prominent in the caudate and putamen, thalamus, medulla, pons and brain stem. It was present in lower relative amounts in the temporal, parietal and occipital lobe and cerebellum. The portion of the brain with the lowest relative amount was the frontal cortex. It was present in brains of stillborn infants, indicating that it was not a developmentally late protein. There was no significant difference in the amount of *Pc 1 A* in grey compared with white matter. Its molecular weight is 19,300 by SDS-gel electrophoresis. Its isoelectric point in 9 M urea is 5.7, comparable to 4.2–4.7 in the absence of urea.

Relation of *Pc 1 Duarte* to neurological disease

To determine if this mutation was related to any specific neurological disease, brains from 276 individuals who had died of various disorders were examined (Table 1). In 103 controls dying of cancer, heart disease, accident and other non-neurological disorders, there were 28 heterozygotes and 4 homozygotes from a total of 32 carrying the *Pc 1 Duarte* mutant. In 49

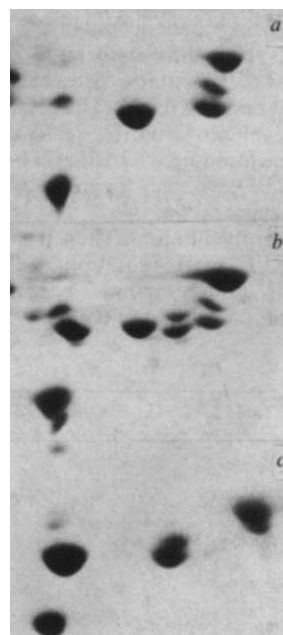


Fig. 2 Critical area of the gel *Pc 1 A/Pc 1 A* homozygous (a), *Pc 1 Duarte/Pc 1 A* heterozygote (b) and *Pc 1 Duarte/Pc 1 Duarte* homozygous (c) individuals. In the heterozygote three polypeptides are split into six.

Table 1 *Pc 1* Duarte in various neurological disorders

Diagnosis	n	D/A	%	D/D	%	Total % carrying mutation
Controls						
Without neurological disease	103	28	27.2	4	3.9	31.1
Huntington disease	49	16	32.7	0	0.0	32.7
Total	152	44	28.9	4	2.6	31.6
Virus-associated diseases						
MS	40	21	52.5	0	0.0	52.5
SSPE	5	2		0		
AMLS	4	3		0		
Total	49	27	55.1	0	0.0	55.1
Alzheimer disease	3	1		0		33.3
Schizophrenia						
Definite schizophrenia	24	9		0		37.5
Probable schizophrenia	6	1		0		14.3
Total	30	10		0		33.3
Depressive disease						
St Louis series						
Suicide, depressive	15	6	40.0	3	20.0	60.0
Suicide, manic depressive	8	4		1		
Suicide, alcoholic	5	3		1		
Total	28	13	46.4	5	17.9	64.2
Other sources						
Suicide	3	1		0		
Manic depressive	3	3		0		
Alcoholic	8	5		0		
Total	14	9	64.3	0	0.0	64.3
Total depressive disease	42	22	52.4	5	11.9	64.3
Total manic depressive	11	7	63.6	1	9.1	72.7

No % given with *n* less than 10. MS, multiple sclerosis; SSPE, subacute sclerosing panencephalitis; AMLS, amyotrophic lateral sclerosis.

individuals with Huntington disease there were 16 heterozygotes and no homozygotes. As this is a single gene disorder and *Pc 1* is obviously not the affected gene, the Huntington disease patients can be added to the other controls to give a total of 152 individuals, with 48, or 31.6%, carrying the mutant gene. In these control individuals the gene frequency for the *Pc 1* A allele, *p*, is $2 \times AA + AD/2N$ or 0.83, and the frequency of the *Pc 1* Duarte allele, *q*, is 0.17. The expected *DD* frequency, q^2 , is 0.029, which was not significantly different from the observed frequency of 0.026.

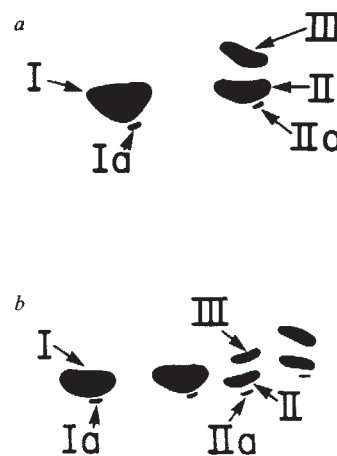
A surprising association was found with multiple sclerosis. Of 40 individuals examined, 21, or 52.5%, were heterozygotes. Comparison with the above controls by the Chi square test gives an χ^2 of 6.0 and $P < 0.025$. Combination of multiple sclerosis with subacute sclerosing panencephalitis and amyotrophic lateral sclerosis, all apparently associated with viral infections²⁰⁻²⁴, gave a total of 27 heterozygotes (55.1%) in 49 cases. Here the χ^2 was 8.77 and $P < 0.005$. The relative risk²⁵ to *Pc 1* Duarte carriers of developing multiple sclerosis is 2.4.

The mean age of the multiple sclerosis *AD* heterozygotes was 50.1 ($n = 20$, s.d. = 13.3), whereas the mean age of the multiple sclerosis *AA* homozygotes was 47.9 ($n = 18$, s.e.m. = 14.2). These are not significantly different. Thus, it cannot be said that the presence of the *Pc 1* Duarte mutation leads to earlier, more severe disease. The frequency among 30 definite and probable schizophrenics was similar to that in controls.

Table 2 χ^2 and *P* values

	χ^2	<i>P</i>
For total % carrying the <i>Pc 1</i> Duarte mutation		
Virus-associated diseases		
Controls versus MS	6.02	<0.025
Controls versus MS+SSPE+AML	8.77	<0.005
Depressive disease		
Controls versus St Louis series	10.89	<0.001
Manic depressive disease	7.70	0.005
Controls versus total depressive disease	14.84	<0.0005
For % <i>DD</i> homozygotes		
Depressive disease		
Controls versus St Louis series	11.54	0.001
Controls versus total depressive disease	6.40	0.01

Because of some preliminary observations suggesting a high frequency of *Pc 1* Duarte in manic-depressive psychoses, alcoholism and suicide, an effort was made to obtain more brain samples from individuals with depressive diseases. I obtained 28 brain samples, part of a collection made over the years from individuals who had committed suicide. Medical and family records were examined and the individuals classified into suicide-alcoholic or suicide-depressive, depending on a set of criteria outlined by Feighner *et al.*²⁶. Those who did not fit this classification or for whom insufficient information was available were not examined. Some of the suicide depressives were further classified as manic-depressive²⁶. The results are given in Table 1. There were no significant differences between the three groups, but in the total group of 28 there were 13 *AD* heterozygotes and 5 *DD* homozygotes. Thus, in this group, 64.2% carried the *Pc 1* Duarte mutation and 17.9% were homozygous. As shown in Table 2, these figures are all significant, and for *DD* homozygotes in controls compared with the St Louis brains, $P = 0.001$. Among suicides, manic depressives and chronic alcoholics from other sources, 9 of 14, or 64%, carried the *Pc 1* Duarte mutation. Of 42 total individuals in these groups, 52.4% were heterozygous *AD* and 11.9% homozygous *DD* for a total of 64.3% carrying the mutation. These are all significantly different from the controls (Table 2). Within the manic-depressive group only, 73% carried the *Pc 1* Duarte mutation. Based

**Fig. 3** Nomenclature for the *Pc 1* A (a) and *Pc 1* Duarte (b) polypeptides.

on the St Louis series, the relative risk of a *DD* homozygote developing depressive disease was 8.0; for the St Louis plus other series it was 5.0.

As the mean age of the non-Huntington disease controls was 52.6 (s.d. 18.5) and the mean age of the *AD* and *DD* individuals with depressive disease was 46.5 (s.d. 16.1), any age correction for the late onset of depressive disease would not increase the frequency of depressives among the mutant individuals.

The data on sex and race for those on whom such information was available are shown in Table 3. As this includes all cases, the total frequency for *Pc 1* Duarte is higher than that in the control group alone. The equal occurrence in males and females indicates that the gene is on an autosome. The equal occurrence in caucasians and blacks indicates that the gene has a widespread distribution and makes it unlikely that the increased frequency in depressive disease and multiple sclerosis is simply due to the presence of a subset of the population having both a high frequency of this gene and increased predisposition to these two disorders.

The genetics of depressive disease

There is a striking similarity between the results obtained here and the model of the genetics of schizophrenia proposed by Kidd

Table 3 Occurrence of *Pc 1* Duarte according to sex and race

	AA	AD	DD	Total	%AD +DD
Males	92	55 (36.4%)	4 (2.6%)	151	39.0
Females	55	37 (39.5%)	4 (4.1%)	96	42.6
Total	147	92	8	247	
Caucasians	93	59 (38.1%)	3 (1.9%)	155	40.0
Blacks	12	9 (40.9%)	1 (4.5%)	22	45.4
Total	105	68	4	177	

and Cavalli-Sforza¹². Although their model was for schizophrenia, the genetic aspects of the depressive disorders are similar. In both, studies of twins show concordance rates for monozygotic twins of 30–93% and for dizygotic twins of 0–24% (refs 3–5). The significantly greater concordance rate for monozygotic compared with dizygotic twins indicates that genetic factors play an important part, and the concordance rates of less than 100% for monozygotic twins indicates that environmental factors are also important. Both have a relatively high frequency in the general population, 0.3–2.9% for schizophrenia³ and 0.6–3.3% for depressive disease^{4,27,28}. Both show a frequency in first-degree relatives of 5–39% (refs 3,4,29), significantly greater than in the general population but less than expected on the basis of a fully penetrant dominant gene.

The basic element of the Kidd-Cavalli-Sforza hypothesis is that a single, primarily recessive gene is involved in combination with a threshold effect due to environmental factors, and the gene frequency is high. I have modified their Fig. 2 to represent AD heterozygotes and DD homozygotes (Fig. 4). According to this model, *Pc 1* Duarte is the single major gene involved in depressive disease; most AA individuals are unaffected but some may be; AD individuals are at greater risk but the majority are unaffected; DD individuals are at greatest risk, but even here some are unaffected. The observed gene frequency is high, 0.17.

In the depressive diseases the gene would be less recessive than in the application of the model to schizophrenia. This conforms with the results of Mendlewicz and Rainer²⁹ in their application of the Kidd-Cavalli-Sforza hypothesis to manic-depressive disease. Depressive disease seems to be at least a partially heterogeneous disorder. The most common distinction is between monopolar (pure depressive) and bipolar (manic depressive) disease, but even here the genetic overlap is considerable^{4,30–33}.

Heterogeneity on the basis of response to drugs has also been suggested^{34,35}. Although the incidence of AD and DD individuals may be even greater in certain types of depressive disease, the subtypes of suicide-depressive, suicide-manic depressive and suicide-alcoholic were similar (Table 1). Of the 11 manic depressives, 9 were male; the two females were AD. The preliminary evidence suggesting a relationship between *Pc 1*

Duarte and chronic alcoholism could explain some of the genetic aspects of this disorder^{36,37} and its association with affective illness³⁸.

Multiple sclerosis

The significant association of *Pc 1* Duarte with multiple sclerosis and with other similar diseases such as subacute sclerosing panencephalitis and amyotrophic lateral sclerosis was intriguing. These diseases are either clearly associated with viruses, as in the case of subacute sclerosing panencephalitis^{21–23}, or suspected of being so associated^{20,24}. Studies of *HLA* genes^{39–42} show an association of *HLA-A1* and 3, *B7*, *Cw2* and *Dw2* with

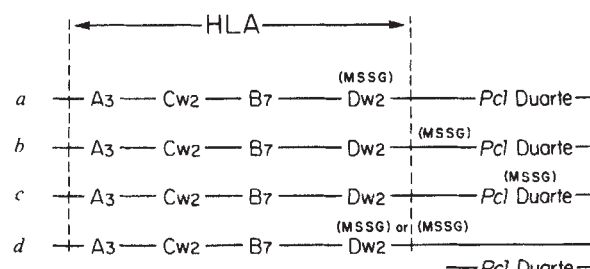


Fig. 5 Possibilities for the linkage relationship between *Pc 1* Duarte, the *HLA* locus and the multiple sclerosis susceptibility gene (*MSSG*). The *MSSG* mutation may have occurred on a chromosome carrying the *HLA* A-3, *Cw2*, *B7*, *Dw2* haplotype. However, these associations with multiple sclerosis do not necessarily imply that these genes are all on the same chromosome.

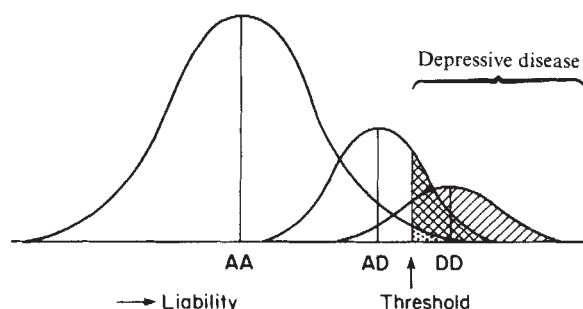
multiple sclerosis, the latter being the most significant. These data have suggested the presence of a multiple sclerosis susceptibility gene (*MSSG*) which is either the same as the *Dw2* gene or linked to it. Figure 5 shows the possible relationship between the *HLA* loci, *MSSG* and *Pc 1* Duarte. *Pc 1* Duarte may be linked to the *HLA* locus but separate from the *MSSG*, which is either identical to *Dw2* or linked to it (a and b). *Pc 1* Duarte itself may be the *MSSG* (c), or it may be unlinked to the *HLA* locus and only fortuitously associated with multiple sclerosis (d). Possibility c suggests that the presence of *Pc 1* A Duarte may alter the host response to central nervous system viral infection. Possibilities a and b suggest that the high frequency of *Pc 1* Duarte in the population may have been due to the fortuitous occurrence of this mutation on an *HLA* haplotype that for other reasons has been selected for. This could explain the persistence of a genetically disadvantageous disease associated with a mental disorder, at a relatively high level in the population.

If the *Pc 1* Duarte mutation is the major single gene in depressive disease and is associated with multiple sclerosis, one might expect to see an association between depressive disease and multiple sclerosis. I am unaware of such an association, although Shapiro *et al.*⁴³ report that a significantly higher percentage of patients with a family history of affective disorders had *HLA-B7* (55%) compared to those without such a family history (19%) ($P = 0.001$). This is consistent with possibilities a, b and c in Fig. 5.

These studies suggest that a similar polymorphic gene interacting with environmental thresholds would account for most schizophrenia. We have examined many different subfractions of the caudate, putamen and neocortex of schizophrenics and have not yet found a comparable mutation.

The most difficult aspect of these studies is obtaining brain specimens. I would be pleased to receive frozen specimens from well documented cases of individuals dying of any behavioural or hereditary neurological disorder, especially schizophrenia, depressive disease, chronic alcoholism, hyperactivity syndrome in children, autism, tuberous sclerosis and the hereditary ataxias. These would be examined both for *Pc 1* Duarte and for other mutant proteins.

Fig. 4 Application of the Kidd-Cavalli-Sforza hypothesis¹² to the present data suggesting that *Pc 1* Duarte is the single major gene involved in depressive disease and acts in conjunction with an environmental threshold effect. See text. □, AA individuals; ▨, AD individuals; ▩, DD individuals.



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Mechanism of β -adrenergic relaxation of smooth muscle

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The mechanism of β -adrenergic relaxation was investigated in isolated smooth muscle cells. β -adrenergic agents stimulate cyclic AMP-dependent phosphorylation, enhance Na^+/K^+ transport and induce relaxation. The stimulation of Na^+/K^+ transport is obligatory for relaxation, and we suggest that this stimulation induces relaxation through enhanced $\text{Na}^+/\text{Ca}^{2+}$ exchange.

It has long been recognised that catecholamines cause relaxation of visceral smooth muscle^{1,2}. Despite intensive studies, the biochemical and physiological events that translate this hormone signal into a change in smooth muscle tone are unknown. In other tissues, however, the events responsible for catecholamine actions are better understood. Since Sutherland and Rall suggested that cyclic AMP may serve as the intracellular mediator of the effects of glucagon and adrenaline^{3,4}, considerable evidence has accumulated indicating that many catecholamine actions, notably those mediated by β -adrenergic receptors⁴, are mediated by protein phosphorylations catalysed by protein kinases. These protein phosphorylations are a consequence of an increase in cyclic AMP brought about by β -adrenergic stimulation of adenylate cyclase. The rise in cyclic AMP activates cyclic AMP-dependent protein kinase, which by phosphorylation of specific proteins, alters cellular functions such as glycogenolysis, lipolysis and ion transport^{5–7}.

An analogous sequence of protein phosphorylations has been suggested to mediate β -adrenergic-induced relaxation of smooth muscle^{8,9}. As most evidence suggests that this relaxation results from a decrease in Ca^{2+} availability^{10,11}, it has been further suggested that the protein phosphorylated by this mechanism either directly or indirectly controls Ca^{2+} distribution^{9,12}. Although this hypothesis is plausible, consid-

eration of the literature reveals that evidence for such a cascade in β -adrenergic-induced relaxation is equivocal for several reasons^{13–15}. First, an increase in cyclic AMP does not always correlate with the relaxation induced by isoprenaline. In addition, drug-induced changes in cyclic AMP-dependent protein kinase have yet to be measured. Finally, uncertainty persists regarding the manner in which β -adrenergic agents affect Ca^{2+} distribution. Studies of isoprenaline action suggest effects on the distribution of Na^+ and K^+ (refs 16–19) as well as of Ca^{2+} (refs 11, 17–23). Thus, it is not known if the effects of isoprenaline on Ca^{2+} distribution are direct or a secondary consequence of the redistribution of other ions.

The contradictions and uncertainties concerning the mechanism of β -adrenergic-induced relaxation of smooth muscle can largely be attributed to problems in interpretation of studies on intact tissue. The heterogeneity of cell types, the electrical and mechanical coupling between individual smooth muscle cells, and the complex extracellular diffusion paths have all imposed serious limitations on such studies. The availability of a preparation of enzymatically disaggregated smooth muscle cells from the stomach muscle of *Bufo marinus* permits a more direct examination of the mechanism of β -adrenergic-induced relaxation. Previous studies have revealed that these smooth muscle cells retain many of their mechanical, ultrastructural, biochemical, pharmacological and electrical properties following isolation^{24–31}. Of particular importance to the present studies was the finding that isoprenaline caused a decrease in the responsiveness to contractile agents²⁶. Because isoprenaline also increases cyclic AMP levels²⁶, we examined the possible links between these two effects of β -adrenergic stimulation. Specifically, we examined whether the link involves cyclic AMP-dependent protein phosphorylation and consequent stimulation of the Na^+/K^+ pump. In addition, we have examined how changes in the Na^+/K^+ pump are linked to changes in contractility.

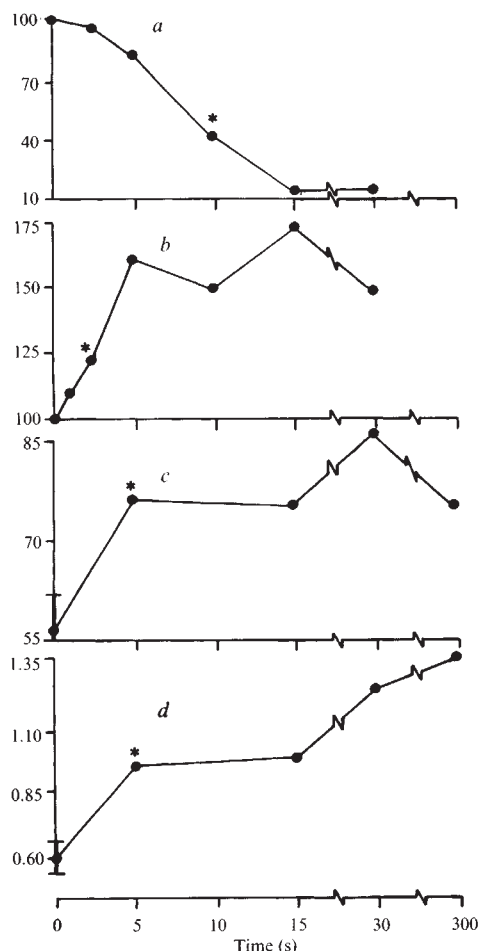


Fig. 1 Time course for the effects of isoprenaline on contractility, cyclic AMP, cyclic AMP-dependent protein kinase and phosphorylase *a* in suspensions of isolated smooth muscle cells. All experiments were carried out at 10^{-5} M isoprenaline. Cells were prepared and suspended as described previously⁴⁷. The asterisk in all panels indicates when the response was significantly different from the control. Statistical significance for each of these four parameters was assessed from data pooled from the following experiments: *a*, % contraction, 12 experiments; *b*, cyclic AMP, 5 experiments; *c*, protein kinase, 4 experiments; *d*, phosphorylase, 4 experiments. *a*, Isoprenaline significantly decreased contractility within 10 s. Changes in the contractile state of a population of cells were assessed by comparing pulse height distributions as drug-treated and untreated aliquots of a single cell suspension were passed through a Coulter counter⁴⁷. This method of analysing contractile changes is based on the finding that pulse amplitude is a measure of cross-sectional area that increases as the cells actively shorten. As the cells in suspension are already relaxed, the addition of inhibitory agents does not result in a shift in pulse height histograms. Therefore, we determined the changes in potential for contraction by first incubating the cells with isoprenaline and then challenging them with a brief (6 s) exposure to the excitatory agent, carbachol (5.5 μ M). The effect of isoprenaline was evident as a progressive decrease in the contractile response to carbachol with longer isoprenaline exposures. It was expressed as a percentage of the contractile response observed in the presence of carbachol alone. As the effect of isoprenaline on all parameters other than contractility was determined without an excitatory agent, it is important to note that previous studies have shown that the time course for isoprenaline action on contractility is unaffected by the use of carbachol. This justifies comparison of the time course of the effects of isoprenaline on contractility determined in the presence of carbachol with the time course of effects on other parameters. *b*, Isoprenaline significantly increased cyclic AMP levels within 2.5 s. Cyclic AMP was determined by competitive protein binding assay⁴⁸ in cell extracts prepared by sonication in perchloric acid. Changes in cyclic AMP were expressed as a percentage of control cyclic AMP levels. *c*, Isoprenaline significantly increased the activity of cyclic AMP-dependent protein kinase within 5 s. Cell extracts were prepared by the addition of NaCl and EDTA to the cell suspension just before homogenisation with a Tekmar omnimixer. The ratio of protein kinase activities assayed in the absence and presence of 4 μ M cyclic AMP was used as an index of the state of enzyme activation *in situ*³³. These values are expressed as % active protein kinase. *d*, Isoprenaline significantly increased phosphorylase *a* activity within 5 s. Cell extracts were prepared by the addition of KF and EDTA to the suspension just before homogenisation with a Tekmar omnimixer and assayed as described by Hardman *et al.*⁴⁹. Activity is expressed as nmol glucose-1-phosphate formed per min per mg protein.

Isoprenaline effects on contractility and cyclic AMP-dependent phosphorylation

A series of experiments was carried out to determine the involvement of cyclic AMP-dependent protein phosphorylation in the change in contractility induced by isoprenaline. The contractile responsiveness of the cells was monitored using the Coulter counter technique (see legend to Fig. 1 and ref. 27). As the cells in suspension were already fully extended, the relaxing effect of isoprenaline could only be assessed as inhibition of the response to an excitatory stimulus. As shown in Fig. 1, isoprenaline decreased the contractile response of isolated smooth muscle cells to excitatory agents in a time-dependent manner. It also increased cyclic AMP levels in the isolated cells (Fig. 1), and both the increase in cyclic AMP and the decrease in contractility exhibited identical dose dependencies²⁶. Both effects are apparently mediated by β -adrenergic receptors, as both responses are fully blocked by propranolol (10^{-4} M). Although both responses occurred rapidly, the increase in cyclic AMP seems to occur more rapidly than the decrease in contractility. Thus, the data are consistent with the notion that relaxation is mediated by a cyclic AMP-dependent process. This is further supported by the finding that cyclic AMP mimicked the effect of isoprenaline on contractility when applied exogenously²⁶ or when applied intracellularly by microinjection²⁹.

To evaluate further the role of cyclic AMP and cyclic AMP-dependent protein phosphorylation in relaxation, we examined the effects of isoprenaline on cyclic AMP-dependent protein kinase. The toad smooth muscle contains a cyclic AMP-dependent protein kinase which, on the basis of its elution pattern from DEAE-Sephadex, is primarily the type II isozyme^{32,33}. The activity of this enzyme was increased by exposure of the cells to isoprenaline (Fig. 1), with an average twofold maximal change. The increase in protein kinase activity seemed to occur more rapidly than the change in contractility. Thus, the data are consistent with the notion that relaxation is mediated by changes in the activity of cyclic AMP-dependent protein kinase.

To gain some insight into the rate at which cyclic AMP-dependent protein phosphorylation occurs in intact cells, we examined the effects of isoprenaline on phosphorylase activity. In many tissues, activation of cyclic AMP-dependent protein kinase leads to activation of phosphorylase by a sequence of protein phosphorylations involving phosphorylase *b* kinase as an intermediate. Isolated smooth muscle cells contain phosphorylase *b* kinase, as extracts catalysed the conversion of purified phosphorylase *b* to phosphorylase *a*. Exposure to isoprenaline caused a nearly twofold increase in phosphorylase *b* kinase activity (control: 0.047 ± 0.006 ; isoprenaline: 0.084 ± 0.0015 U phosphorylase *a* formed per min per mg protein; assayed as described by Bueding *et al.*³⁴). Isoprenaline also increased phosphorylase *a* activity (Fig. 1) and dibutyl cyclic AMP mimicked this effect. Like the activation of cyclic AMP-dependent protein kinase, activation of phosphorylase by isoprenaline occurred rapidly (significant increases were observed within 5 s). Thus, cyclic AMP-dependent protein phosphorylation can occur rapidly enough in the intact cell to mediate β -adrenergic-induced relaxation. It seemed unlikely, however, that the change in phosphorylase *per se* could account for relaxation³⁴. Therefore, we investigated whether other cellular processes are altered by isoprenaline and if so, whether such alterations involve cyclic AMP-dependent protein phosphorylation.

Isoprenaline effects on Na^+ and K^+ fluxes

Several previous studies had suggested that β -adrenergic effects on smooth muscle might involve a redistribution of Na^+ and K^+ across the cell membrane²⁰⁻²³. Because of the difficulties imposed by intact tissue, these data are somewhat equivocal and inconclusive. Therefore, we investigated the effect of isoprenaline on unidirectional Na^+ and K^+ fluxes in the isolated

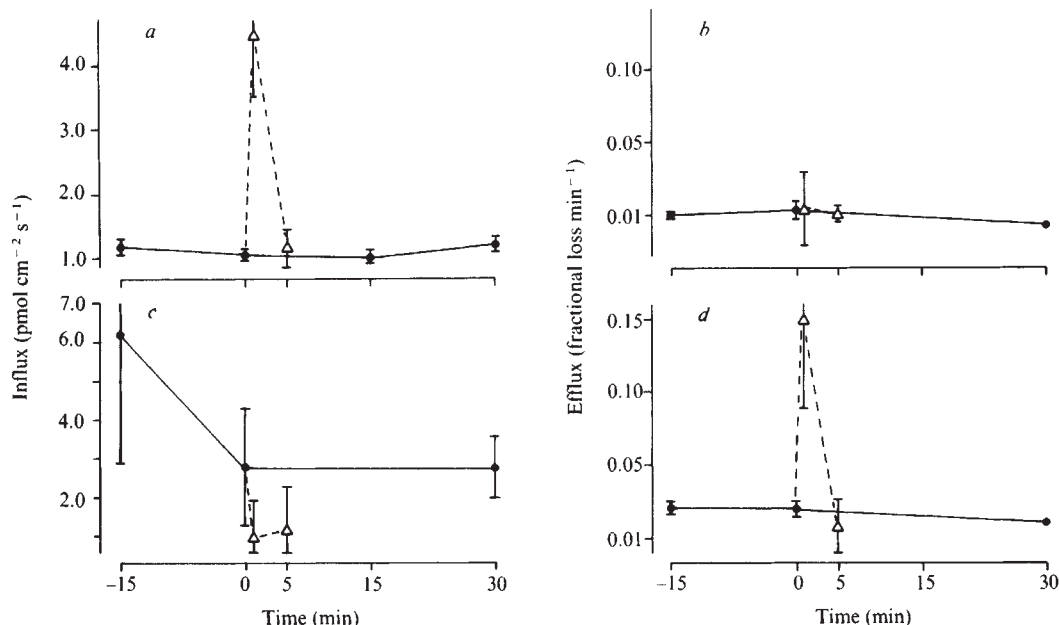


Fig. 2 Effects of isoprenaline on the influx and efflux of Na^+ and K^+ in isolated smooth muscle cells. For influx studies, cells in aliquots of a single suspension were centrifuged through silicone oil at various times after addition of radioactive ion. ^{14}C -urea and ^3H -inulin were included to determine the total water and extracellular water associated with the cell pellet, respectively. Cellular ion uptake (expressed as $\text{pmol cm}^{-2} \text{s}^{-1}$) after correction for extracellular radioactive ion, and backflux and decay of radioactive ion during counting, was calculated⁵⁰ using an average surface area/volume ratio of $5 \times 10^3 \text{ cm}^{-1}$ obtained by morphometric analysis of fixed cells. The same general procedure was used to study ion efflux. After several hours' exposure to radioactive ion, the specific activity of the bathing medium was reduced by dilution and the rate of loss of radioactivity from the intracellular compartment was monitored. The ion content of the cells was determined by flame photometry. An index of efflux rate was obtained by calculating⁵⁰ the fractional change in specific activity per min. In both influx and efflux experiments, isoprenaline (10^{-4} M) was added after the fluxes had achieved a stable rate, indicating exchange between a simple two-compartment system. The addition of isoprenaline was denoted time zero so that experiments could be compared. The cells did not seem to have been damaged by the experimental procedures, as the K^+ content was essentially identical to that in the intact tissue (cells: $131.5 \pm 5.2 \text{ mmol per l}$ intracellular water; tissues $138.1 \pm 4.5 \text{ mmol per l}$ intracellular water). Each point on the graph represents the mean $\pm$ s.e.m. calculated from the following experiments. **a**, ^{42}K influx. The addition of isoproterenol at time zero stimulated ^{42}K influx 3.4 fold (triangles) over control (circles). This effect was observed within 1 minute but was no longer evident at 5 min. **b**, ^{42}K efflux. The addition of isoproterenol (triangles) at time zero did not stimulate the efflux of ^{42}K above that of controls (circles). **c**, ^{24}Na efflux. The addition of isoproterenol (triangles) at time zero stimulated ^{24}Na efflux 7.6 fold over that of the control (circles). Like the effects of isoproterenol on ^{42}K influx, this effect was observed within 1 minute, but was no longer evident by 5 minutes. **d**, ^{24}Na influx. The addition of isoproterenol (triangles) at time zero did not stimulate ^{24}Na influx over control (circles). The influx values for the isoproterenol group represent an underestimate of the true values in that the backflux correction factors could not fully correct for the enhanced backflux (efflux) caused by isoproterenol.

cells. Exposure to this drug caused a large, rapid increase in ^{42}K influx and ^{24}Na efflux (Fig. 2). The stimulation was maximal at the earliest time which could be investigated (45 s) and was detectable at concentrations as low as 10^{-8} M . At concentrations of 10^{-6} M or greater, ^{42}K influx was increased by an average of 3.4 times above the control value of $1.2 \pm 0.3 \text{ pmol cm}^{-2} \text{s}^{-1}$. ^{24}Na efflux was increased by 7.6 times above its control value of $2.9 \pm 0.6 \text{ pmol cm}^{-2} \text{s}^{-1}$. By contrast, ^{42}K efflux and ^{24}Na influx were not significantly altered by isoprenaline. As the stimulation of ^{24}Na influx and ^{42}K efflux was no

longer detectable by 5 min, large changes in ion content in response to isoprenaline are not to be expected. We calculate a net Na^+ efflux per cell in response to isoprenaline of 0.08 pmol ; this represents a net decline in intracellular Na^+ of only 8%. The predicted change in K^+ content would be less than 1%. Given the analytical error associated with ion content determinations, it is not surprising that isoprenaline produced no significant change in either Na^+ ($65.5 \pm 4.4 \text{ mmol per l}$ intracellular water) or K^+ ($131.5 \pm 5.2 \text{ mmol per l}$ intracellular water) content. The observation that isoprenaline stimulated the movement of Na^+ and K^+ against their respective electrochemical gradients suggested that the Na^+/K^+ pump had been stimulated. To test this possibility, we examined the effects of ouabain on K^+ movement. Ouabain at $5 \times 10^{-4} \text{ M}$ completely abolished the stimulatory effect of isoproterenol on ^{42}K influx in three experiments; at this dose it reduced the control ^{42}K uptake by 50% (data not shown). These flux studies indicate thus that isoprenaline stimulates the Na^+/K^+ pump in the isolated smooth muscle cells. Two important questions arise from this conclusion. First, is the change in pump activity mediated by a cyclic AMP-dependent phosphorylation mechanism as has been proposed for relaxation? Second, does the change in pump activity lead to relaxation, and if so, how?

Table 1 Stimulation of Na^+/K^+ -dependent ATPase activity by cyclic AMP-dependent protein kinase

	Na^+/K^+ ATPase activity (nmol per min per mg protein)
- Protein kinase	40 ± 17
+ Protein kinase	81 ± 28

Membrane fragments (50–200 μg) as described in the legend to Fig. 3 were preincubated with 10–50 μg of partially purified cyclic AMP-dependent protein kinase⁵¹ (specific activity of 25 pmol histone phosphate formed per min per μg protein). As the protein kinase preparation had appreciable catalytic activity even in the absence of cyclic AMP, the nucleotide was not included. The reaction mixture was diluted and ATPase activity determined by the liberation of ^{32}P from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (ref. 52). The difference in ATPase activity assayed in the presence of K^+ alone and in the presence of $\text{Na}^+ + \text{K}^+$ was defined as Na^+/K^+ ATPase activity. Preincubation of fragments with protein kinase and $\text{ATP} \cdot \text{Mg}^{2+}$ increased Na^+/K^+ ATPase activity by an average of twofold (values above represent the mean $\pm$ s.e.m. for 8 experiments). The extent of activation was 63–180%; in 1 experiment no effect was observed. The activation of Na^+/K^+ ATPase activity was statistically significant ($P < 0.05$, paired analysis). ATPase activity assayed in the absence of Na^+ was not significantly altered by protein kinase (0.194 ± 0.07 compared with $0.185 \pm 0.06 \mu\text{mol per min per mg}$). The Na^+/K^+ ATPase activity of membrane fragments exhibited several characteristics^{53,54} of a Na^+/K^+ pump; inhibition by ouabain (10^{-3} M), F^- and Be^{2+} ; low apparent K_m for ATP ($< 50 \mu\text{M}$); stability to mild treatment with SDS; ability to form an acid-stable, base-labile phosphoprotein; turnover number of 10^4 min^{-1} calculated from phosphoprotein and ATPase values.

Cyclic AMP-dependent stimulation of the Na^+/K^+ ATPase

A series of experiments was carried out to determine the manner in which isoprenaline affects Na^+/K^+ pump activity. The effects on the pump apparently result from interaction with a β -receptor, as the stimulation of ^{42}K influx by isoprenaline (10^{-5} M) was fully blocked by 10^{-6} M pindolol. Furthermore, the effects on the pump seem to be mediated by an increase in cyclic AMP, as dibutyryl cyclic AMP (10^{-4} M) caused an

increase in ^{42}K influx identical in both magnitude and time course to that produced by isoprenaline (data not shown).

As protein kinases have been reported to affect Na^+/K^+ ATPase activity in other systems³⁵⁻³⁸, we investigated the possibility that Na^+/K^+ ATPase may be stimulated as a consequence of cyclic AMP-dependent protein phosphorylation. Membrane fragments prepared from isolated smooth muscle cells contain an ATPase activity dependent on the presence of both Na^+ and K^+ which has characteristics similar to 'sodium pump' ATPases of other tissues (Table 1). Reacting these membrane fragments with cyclic AMP-dependent protein kinase increased ATPase activity (Table 1, Fig. 3). As seen in Fig. 3, the extent of ATPase stimulation approached a maximal value with increasing amounts of protein kinase. This stimulatory effect of protein kinase seems to result from a change in the Na^+/K^+ ATPase, as activity assayed in the absence of Na^+ was not affected (Table 1). Finally, the stimulation was blocked by protein kinase inhibitory protein, suggesting that the change in Na^+/K^+ ATPase specifically results from phosphorylation catalysed by the protein kinase (Fig. 3).

Na^+/K^+ transport and relaxation

Experiments were carried out to investigate the coupling between the cyclic AMP-dependent stimulation of Na^+/K^+ transport and the cyclic AMP-dependent relaxation. To assess whether these two events are causally linked, we examined the effect of isoprenaline on contractility in the presence of ouabain or in K^+ -free medium, conditions in which Na^+/K^+ transport should be limited. In our standard medium containing 3 mM K^+ , isoprenaline inhibited the contractile response to carbachol by 53%; however, when the cells were resuspended in nominally K^+ -free medium just before exposure to isoprenaline, only a 16% inhibition was observed (Table 2). However, removal of external potassium had no effect on the response to carbachol. An even more striking attenuation of relaxation was observed when ouabain was used to limit Na^+/K^+ transport (Table 2).

Table 2 Effects of changes in extracellular K^+ and ouabain on the response to isoprenaline

Cell suspension medium	% Inhibition of contractility by isoprenaline	No. of experiments
Amphibian physiological saline (APS)	50 ± 15	3
APS + ouabain (5×10^{-4} M)	-27 ± 32	
APS	53 ± 8	
K^+ -free APS	16 ± 23	5
APS	30 ± 7	
90 mM K^+ /APS	43 ± 16	

The inhibitory effects of (10^{-5} M) isoprenaline on the contractile responses to carbachol (10^{-6} – 10^{-4} M) were compared in aliquots of cells isolated as a group, but then divided so that half were suspended in normal amphibian physiological saline (3 mM K^+) and the others were suspended in modified amphibian physiological saline²⁵. K^+ concentration changes were effected by molar substitution of K^+ salts for Na^+ salts. For the groups containing ouabain or K^+ -free medium the effect of isoprenaline was determined within 30 s of accomplishing the change from APS to the modified medium; this protocol was chosen to minimise changes in ion gradients which would eventually follow inhibition of the Na^+/K^+ pump. For the cells exposed to 90 mM K^+ , the effect of isoprenaline was determined 15 min after raising the extracellular K^+ ; this period of time was chosen to allow the cells to relax following the initial phasic contraction induced by exposure to high K^+ levels. To test for the inhibitory effect of isoprenaline on contractility, aliquots were preincubated with isoprenaline (10^{-5} M) for 9 s before testing responsiveness to a 6-s carbachol exposure. Contractility was assessed as described in Fig. 1. Intracellular microelectrode recordings reveal that increasing the extracellular K^+ to 90 mM causes a sustained, almost complete depolarisation³¹.

Thus, an increase in Na^+/K^+ pumping seems to be essential to the isoprenaline-induced relaxation.

We next considered the manner in which an increase in Na^+/K^+ pump activity might lead to a decrease in Ca^{2+} availability and thus relaxation. Two hypotheses are consistent

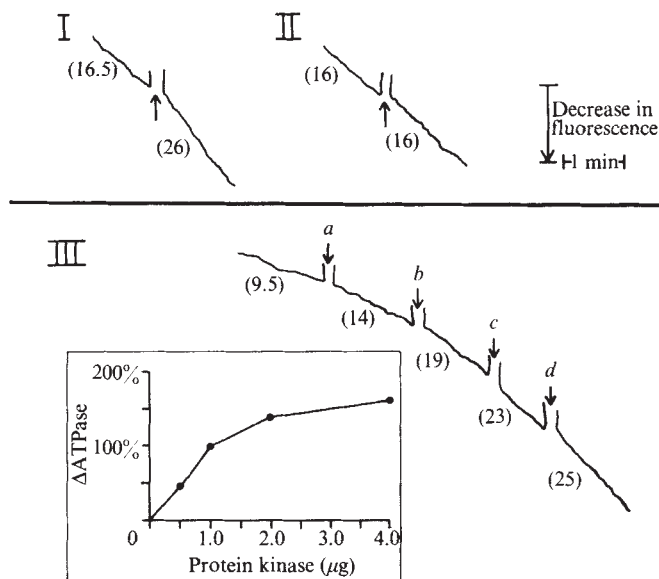


Fig. 3 Stimulation of ATP hydrolysis by cyclic AMP-dependent protein kinase. Membrane fragments were prepared from isolated smooth muscle cells by differential centrifugation of extracts prepared in sucrose (0.25 mM), imidazole (50 mM) and EDTA (10 mM) buffer, pH 7.2. Fragments sedimenting between 1.2×10^3 g and 4×10^3 g were assayed for ATPase activity fluorometrically⁵⁵. ADP formation was followed as the decrease in fluorescence on conversion of NADH to its oxidised form, the rate of change being indicated in parentheses. Vertical bar represents 5 nmol of NAD^+ . In traces I and II, 10 μg of membrane fragments were used. The effects of cyclic AMP-dependent protein kinase (2 μg added at arrow) were tested in the absence (I) and presence (II) of cyclic AMP-dependent protein kinase inhibitory protein⁵⁶ added before the membrane fragments. III, 20 μg of membrane fragments (a different preparation from that used in I and II) was assayed in the presence of increasing amounts of protein kinase. a, 0.5 μg protein kinase was added; b, additional 0.5 μg ; c, additional 1.0 μg ; d, additional 2.0 μg . Note that protein kinase stimulation of ATPase activity seems to be saturable (see insert). Control studies revealed that the stimulation of ATPase activity could not be accounted for by ATPase activity of the protein kinase or by protein kinase-induced alterations in the activity of the coupling enzymes (pyruvate kinase and lactate dehydrogenase) used in the assay. It is unlikely that the stimulation of ATP hydrolysis represents a rapid turnover of a phosphoprotein in the membrane fragments catalysed by the protein kinase and a membrane-associated phosphoprotein phosphatase, as maximal catalytic activity of protein kinase could produce less than 10% of the observed stimulation of ATP hydrolysis.

with our present understanding of Ca^{2+} movement in smooth muscle. The first is that a decrease in calcium permeability might result from the hyperpolarisation which accompanies increased Na^+/K^+ pump activity³⁹⁻⁴². Alternatively, the decrease in internal Na^+ generated by increased Na^+/K^+ pump activity might accelerate $\text{Na}^+/\text{Ca}^{2+}$ exchange in the plasma membrane and/or sarcoplasmic reticulum⁴³⁻⁴⁶. Although our understanding of the link between Na^+/K^+ pump activity and relaxation is incomplete, a change in membrane potential does not seem to be essential for isoprenaline-induced relaxation. This follows from the observation that the inhibitory effect of isoprenaline was unaffected by depolarisation of the cells with 90 mM K^+ (Table 2).

We are thus left with the possibility that increased $\text{Na}^+/\text{Ca}^{2+}$ exchange is responsible for linking Na^+/K^+ pump activity with relaxation. Experiments in which extracellular Na^+ was altered suggest that $\text{Na}^+/\text{Ca}^{2+}$ exchange may be important for the control of intracellular Ca^{2+} in these smooth muscle cells, as it is in other smooth muscle preparations⁴³⁻⁴⁶. Specifically, exposure of cells to Na^+ -free medium caused contraction. The $t_{1/2}$ for this response averaged 15 min (four experiments) and the magnitude of the observed contraction was equivalent to the maximal contractile response obtained with carbachol. These data suggest that Ca^{2+} sequestration depends on the Na^+ gradient, and that a change in this gradient produced by stimulation of the Na^+/K^+ pump may lead to relaxation.

Model for isoprenaline action

A model for the mechanism underlying the relaxation induced by isoproterenol is shown schematically in Fig. 4. This hypothesis accounts for all of the observations on isolated smooth muscle cells. This scheme also includes what we believe to be the most plausible means by which stimulation of the Na^+/K^+ pump leads to relaxation. The essential features of the scheme are: (1) isoprenaline binds to the β -adrenergic receptor, causing a rapid increase in intracellular cyclic AMP which in turn activates cyclic AMP-dependent protein kinase; (2) the increased activity of

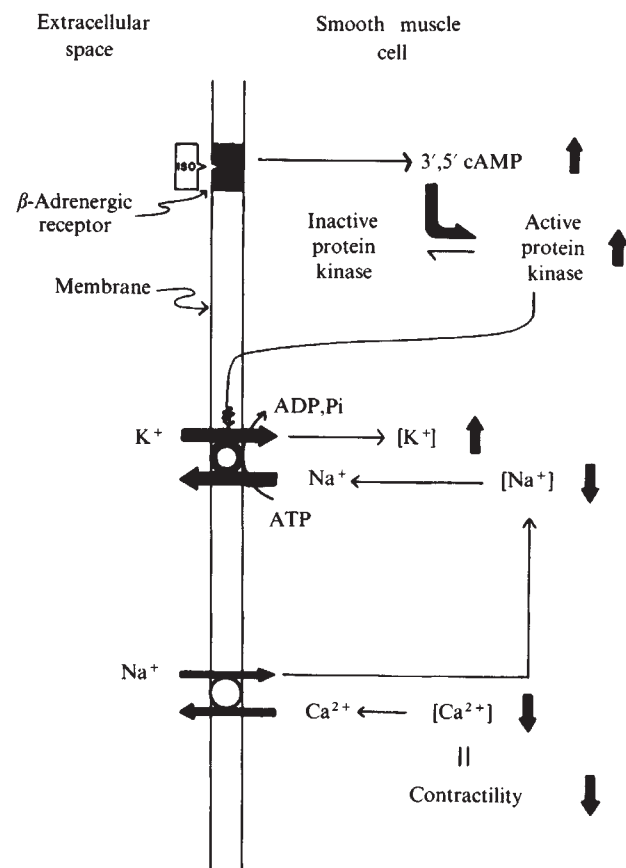


Fig. 4 Scheme for β -adrenergic relaxation of smooth muscle. It is proposed that β -adrenergic agents stimulate the Na^+/K^+ pump through cyclic AMP-dependent protein phosphorylation, and thereby produce relaxation. Although the link between enhanced pump activity and relaxation has not been fully explored, the involvement of a $\text{Na}^+/\text{Ca}^{2+}$ exchange process seems to be most reasonable. The model shows $\text{Na}^+/\text{Ca}^{2+}$ exchange accelerated at the plasma membrane, but a similar $\text{Na}^+/\text{Ca}^{2+}$ exchange system in the sarcoplasmic reticulum and/or mitochondrial membranes might also contribute to the observed decrease in Ca^{2+} availability. Further details regarding this scheme and the evidence supporting are given in the text.

protein kinase results in phosphorylation of a protein associated with the Na^+/K^+ ATPase, thus increasing pump activity; (3) as a consequence of increased pump activity, intracellular K^+ rises and intracellular Na^+ falls; (4) the increased Na^+ gradient would accelerate the rate of $\text{Na}^+/\text{Ca}^{2+}$ exchange, resulting in increased Ca^{2+} efflux from the cytoplasm; (5) the change in Ca^{2+} distribution would decrease contractility.

The above description indicates the interdependence of cellular processes which are affected by isoprenaline. Implicit in this scheme is a specific kinetic relationship between these events. First, the changes shown in Fig. 4 will be initiated in a sequential manner beginning with an increase in cyclic AMP. Second, the changes will induce a persistent activation of the Na^+ pump such that relaxation is maintained. This activation

initially elicits large changes in Na^+ and K^+ fluxes, but the resultant changes in ion gradients soon slow pump activity towards basal levels. Similar kinetics for enhanced $\text{Na}^+/\text{Ca}^{2+}$ exchange would also be expected. The large rapid increases in ion movements would rapidly bring the smooth muscle cell to a more relaxed state. Relaxation could then be maintained by only a small persistent increase in Na^+/K^+ and $\text{Na}^+/\text{Ca}^{2+}$ transport processes.

It seems likely that the mechanism outlined above plays a central part in β -adrenergic relaxation of other types of smooth muscle, although additional factors may also be involved. Tests for the involvement of our proposed mechanism in other tissues will probably require the use of isolated cells derived from each tissue type, so that the brief, large changes in ion movements will not be obscured by the diffusion delays and ion binding which complicate studies in intact tissue.

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Structure of the backbone in myosin filaments of muscle

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New X-ray data suggest how myosin rods, themselves α -helical coiled coils, form the thick filament backbone of crustacean muscles by additional supercoiling. Natural transformations of this structure may describe the myosin backbone in many other animals also.

THE thick filaments of striated muscle are remarkable bipolar structures. The α -helical rod portions of the myosin molecules are packed densely in the backbone, yet the enzymatic portions (pairs of 'heads' or 'cross-bridges') lie at the filament surface where they can participate in the tension-generating interaction with actin^{1,2}. While the ordered helical array of relaxed cross-bridges has been described for several muscles, the internal architecture that defines it is unknown: neither X-ray diffraction nor electron microscopy has revealed the structure of the backbone. Studies of the overlaps between molecules in aggregates of myosin subfragments formed *in vitro* have suggested likely packing arrangements of the myosin rods in native filaments³⁻⁵. Perhaps the most attractive of such models are those in which all myosin molecules have identical environments and are thus 'equivalent' to each other⁶. Knowledge of backbone structures in different muscles will help in understanding the design problem of accommodating both the structural and the enzymatic functions of myosin.

In this article, I discuss thick filament structure in the light of new X-ray diffraction data from crustacean muscles. The X-ray patterns include myosin reflections intermediate between the small-angle diffraction due to the cross-bridge array and the wide-angle diffraction from the α -helical polypeptide conformation in the backbone. These reflections suggest that the backbone has a simple cable-like structure, and indicate its relation to the surface array of bridges. Myosin filament structure in other muscles is discussed briefly in terms of plausible transformations of the structure described for the crustaceans.

Symmetry and polymorphism of the surface lattice

In the fast muscles of decapod crustaceans⁷, the thick and thin filaments form a lattice resembling that in insect flight muscles, and the diffraction from the thin filaments has been discussed previously⁸. The myosin filaments are not held in precise transverse register as they are in some insect muscles, and their diffraction is unsampled. Reflections due to myosin in the small-angle X-ray pattern from a relaxed fast lobster muscle (Fig. 1a) include a strong meridional reflection at 145 Å with an off-meridional subsidiary maximum, and non-meridional layer lines at 308 Å and 274 Å. All these reflections become weaker in rigor, when bridges are displaced from their ordered positions round the thick filament and attach to actin. The 145-Å layer line indicates that cross-bridges are located at 145-Å intervals along the thick filament, as found for many other muscles. The non-meridional layer lines reflect the screw symmetry, or rotation between adjacent 145-Å levels (just as frog muscle patterns, for example, indicate a screw symmetry described by

giving the helical repeat of the most prominent cross-bridge helix as 429 Å (ref. 9)). The screw symmetry indicated by the lobster pattern in Fig. 1a differs from that in muscles studied hitherto, the myosin helical repeat being 308 Å. The black spots in Fig. 3a illustrate this surface symmetry. The X-ray pattern is much simpler than that from frog muscles, where certain 'forbidden' reflections⁹ may indicate a surface lattice in which structure units are not perfectly equivalent.

The cross-bridge reflections reveal the screw symmetry very precisely, but derivation of rotational symmetry (the number (*N*) of structure units or myosin molecules per 145-Å level) is less easy. This is because the diffraction depends mainly on the bridge conformation and on the intrinsic form of the surface lattice, and is changed only slightly by increase of *N* with proportional increase in radius from the filament axis¹⁰. (A preliminary study of lobster muscles¹⁰ noted that the layer lines resemble those given by insect flight muscles¹¹ in intensity and

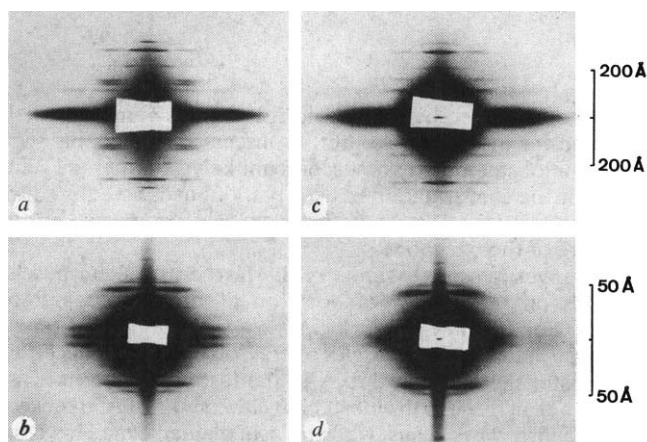
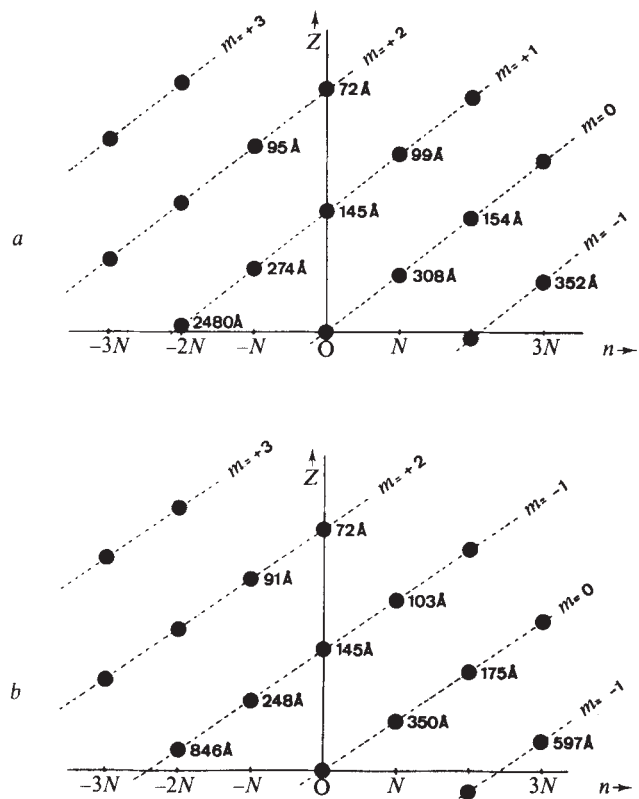


Fig. 1 X-ray diffraction patterns from crustacean muscles, chemically skinned⁸ and examined in the relaxed state in a solution containing 90 mM KCl, 8 mM MgCl₂, 2 mM EGTA, 10 mM histidine, 2 mM Na₂ATP, 1 mM NaN₃, pH 7.0 at 4 °C. X-ray patterns were obtained using conventional mirror-monochromator cameras⁹ and specimen-film distances of 36 cm (a, c) and 12 cm (b, d). a, b, Patterns from a fast abdominal flexor of the lobster. a is the small-angle pattern, showing actin layer lines at 387, 194 and 129 Å, and myosin layer lines at 308, 274 and 145 Å. The actin layer lines were enhanced, and these myosin layer lines weakened, in patterns from the same muscles in rigor. b is a more intense medium-angle pattern. Actin layer lines at 59 and 51 Å are prominent; the conspicuous layer line of axial spacing about 350 Å remained unchanged in the rigor pattern. Certain weak reflections in the small-angle pattern were also unchanged in rigor, such as a subsidiary maximum of the strong layer line at 380 Å and a layer line at 95 Å. c, d, Patterns from a slow muscle of the lobster crusher claw. Slow muscles gave patterns with a higher diffuse background than fast muscles, but layer lines corresponding to those from fast muscles were clearly visible. The small-angle off-meridional reflections occurred at 350 and 248 Å, and the myosin layer line which was unchanged in rigor fell at about 600 Å.

Fig. 2 Diagrams of myosin layer lines. *a*, General representation of the diffraction expected from a helical array with the symmetry being indicated by the myosin diffraction in Fig. 1*a*. Axial positions (Z) of expected layer lines are plotted against their Bessel function orders (n); reflections can be referred to by n and by the value of m assigned to the dotted lines (in the convention of helix diffraction theory²³), and certain axial spacings are indicated numerically. Individual nodes are represented in the observed diffraction by reflections whose radial positions depend on the radii from the filament axis of the structures generating them. The diffraction in Fig. 1*a* is interpreted through the diagram as follows. The strong layer lines at 308, 274 and 145 Å originate from the surface array of cross-bridges, and generate the innermost reflections ($m=0, n=N$), ($m=1, n=-N$) and ($m=1, n=0$) respectively. Bridges do not contribute significantly to any wider-angle reflections, which correspond to other sets of helical tracks through the surface lattice. The reflection $(-1, 3N)$ at 352 Å has special significance, as a strong layer line at this spacing is due to subfilaments interconnecting the surface lattice; the value $n=3N$ corresponds to there being $3N$ subfilaments. (The backbone is not formed from smooth strands; other variations of density, distributed in conformity with the myosin surface lattice, cause weak diffraction at other nodes, such as a subsidiary peak on the 308 Å layer line ($0, N$) and presumably part of the meridional intensities at orders of 145 Å.) Many reflections thus include contributions from both bridges and backbone, located at different radii from the filament axis, and only in special cases will profiles be interpretable in terms of diffraction from simple structures. *b*, Same diagram for the slow muscles. N here takes a different value. These diagrams represent two stages in the continuous transformation of the diffraction that would accompany progressive twisting of the filament. The various fast crustacean muscles could be represented by a series of diagrams with spacings close to those in (*a*). The spacing of the $(-1, 3N)$ reflection equals the helical repeat of the subfilaments in each case. Generally, such diagrams can describe the diffraction from any helical cross-bridge array. Untwisting the filament slightly beyond state (*b*), the spacing of $(0, N)$ reaches 385 Å as observed for insect muscle¹¹, and $(-1, 3N)$ then has a spacing of 1150 Å. Untwisting further, until $(0, N)$ falls at 435 Å, the screw symmetry becomes that observed for frog muscles⁹. $(-1, 3N)$ lies on the equator in the latter case: thus, if the filament were formed from subfilaments homologous to those of crustacean muscles, these would run parallel to the filament axis.



might be explained by the sixfold rotational symmetry widely accepted for insect muscle: but, as discussed below, this symmetry for lobster muscles now seems unlikely.) Neither the value of N nor the conformation of each pair of heads is required for discussing the relationship between myosin symmetry and the structure of the backbone.

The myosin screw symmetry in fast crustacean muscles depends on the anatomical location of the muscle and on species. For example, the principal cross-bridge helical repeat was 300 Å in many fast muscles of crayfish and 308 Å in certain fast lobster muscles, but 310 Å in the fast muscles of crayfish legs, 315 Å in those of the lobster left claw, and 318 Å in those of both crayfish claws. Thus, while myosin filaments may have the same screw symmetry in the skeletal muscles of all vertebrate animals, the fast muscles even of one crustacean species present a family of non-integral myosin helices differing slightly in filament twist.

Diffraction from the myosin backbone

The diffraction expected from a representative member of this family is summarised in Fig. 2*a*. The observed cross-bridge reflections (at 308 Å, 276 Å and 145 Å) represent few of the full set that would result from a perfectly ordered helical array, and this paucity of diffraction indicates that the bridges are considerably disordered about their average positions. More intense patterns from the crustacean muscles did show other myosin reflections, but these were not due to bridges, as they were unchanged in patterns from rigor muscles. The most prominent was a layer line (Fig. 1*b*) whose spacing varied with the screw symmetry of the filament so as always to correspond to that of one particular outer reflection (at 352 Å in the case depicted by Fig. 2*a*). The characteristic radial profile of this layer line is shown in Fig. 4*a*.

Reflections unaffected by the transition to rigor but occurring at spacings related to those of the myosin surface lattice are expected from the myosin backbone and may reveal its structure. First, as different nodes in Fig. 2 correspond to different sets of helical tracks through the surface lattice, the prominence of one particular layer line means that the structure can, at low resolution, be considered as comprising uniform helical strands following one particular direction. Second, the profile of this layer line is characteristic of a cable-like structure whose density is concentrated near a single radius, and whose strands have a diameter of about 40 Å and are packed closely with their neighbours (Fig. 4*b*). The backbone is shown in Fig. 3*a* as a cable whose strands connect up the cross-bridge surface lattice in the direction corresponding to the prominent layer line from the backbone. This structure, apparently identical in relaxed and rigor states, could exist over most of each half of the myosin filament, but will not describe the packing at the ends and centre. Its absolute hand is unknown and is drawn arbitrarily. The hollow core is in agreement with the appearance of a weakly staining centre in crustacean filaments⁷; this observed appearance is variable, perhaps because the core contains small but variable amounts of paramyosin (whose organisation is not revealed by the present X-ray data).

The relationship of cross-bridge positions to the backbone in Fig. 3*a* is such that, with N myosin molecules per 145 Å along the filament, there are $3N$ subfilaments. (The value $N=4$, as drawn, seems the most probable if the tubular appearance of Fig. 3*a* is identified with that in electron microscope images, because the number ($3N$) of subfilaments, separated-centre-to-centre by 40 Å as indicated by the X-ray pattern, must be about 12 to generate the observed⁷ outer diameter of about 200 Å.) The X-ray patterns also contain information about the packing of myosin rods within individual subfilaments. But the 40-Å subfilament diameter directly suggests a cylindrical group of

three myosin rods (each a 2-stranded α -helical coiled coil). Moreover, the repeat length along each individual subfilament in Fig. 3a is 435 Å, so the length of the crustacean myosin rod¹², slightly more than 3×435 Å, would likewise suggest that three overlapping rods comprise each subfilament over most of its length. The myosin rods forming each subfilament must twist round each other if all the myosin heads are accessible to the filament surface.

Diversity of crustacean filaments

The variation of screw symmetry was the only indication in the X-ray patterns that myosin structure in fast crustacean muscles is polymorphic. It corresponds to a slight variation in the angle at which the subfilaments twist round the filament. The versatility of the packing is further illustrated by the slow muscles of the crustaceans. Here the thick filaments have a larger (280 Å) diameter⁷, reflected in the X-ray pattern (Fig. 1c) by, for example, a different profile of the 145-Å layer line. But although the two off-meridional cross-bridge reflections indicate a cross-bridge helical repeat of 350 Å, and the layer line from the subfilaments has an axial spacing of about 600 Å (Fig. 1d), the intensities and profiles of these layer lines are not greatly changed from those in the several fast muscles. Thus an essentially invariant relationship of relaxed cross-bridges to backbone subfilaments seems to be intrinsic in the surface lattice of the fast and slow muscle myosin filaments. The slow muscles differ

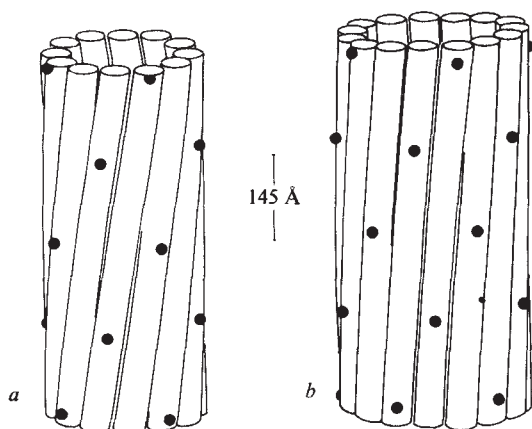


Fig. 3 Relationship of cross-bridge positions to subfilament organisation. *a*, Black spots represent the positions of cross-bridges, as indicated by those reflections in Fig. 1a which weaken in rigor. Further aspects of bridge arrangement, such as radial position and conformation, are not discussed here. The backbone is shown as strands connecting this lattice in the helical direction corresponding to the prominent backbone layer line; subfilament diameter and separation were derived from this layer line (Fig. 4). The several fast crustacean muscles examined would have uniform rotational symmetry, but slightly different screw symmetries. *b*, Similar drawing for slow muscle myosin filaments. Both the rotational and the screw symmetry differ from (*a*). The essentially conserved relationship between bridges and subfilaments probably extends to finer details of the structure than are indicated here.

merely in a smaller twist of the subfilaments round the filament, and in higher rotational symmetry (probably increased from four- to fivefold (Fig. 3b)).

Subfilament structure of myosin filaments

These models for myosin filaments are of a type discussed by Cohen⁶. Re-emphasising that the filaments might be constructed so that all myosin molecules are equivalent, Squire^{5,13} has enumerated such packing arrangements more fully. The fast and

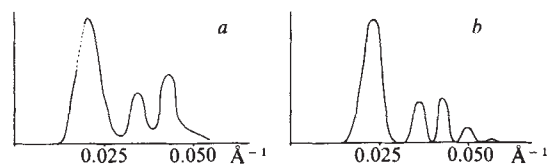


Fig. 4 *a*, Observed radial profile of the strong layer line at 352 Å, indexed as $(-1, 3N)$ in Fig. 2a. Dashed line indicates uncertainty due to incomplete separation from nearby layer lines. *b*, Intensity calculated²⁴ for the layer line at 352 Å from the cable-like backbone in Fig. 3a, normalised to same peak height as (*a*). Subfilaments, centred at 84 Å radius, were each represented by 3 uniformly intertwining myosin rods (smooth cylinders of 8.5 Å radius) centred 12.6 Å from their local axis. The 352-Å layer line is the 'pitch' layer line of the 'major helix'²⁴, and is by far the strongest of the layer lines from the structure. In terms of Bessel functions (J), the profile is given by

$$[J_{12}(2\pi \times 84R)J_0(2\pi \times 12.6R)J_1(2\pi \times 8.5R)/(2\pi \times 8.5R)]^2$$

Cables with more or fewer subfilaments, but with the same (~ 40 Å) separation between neighbours, give similar profiles: but the 12-fold symmetry appears the most probable (see text).

slow muscles of crustaceans illustrate a central point of Squire's general thesis: that whatever the local packing relationships of myosin rods, the unit cell of the surface lattice may change little if the cross-bridge arrangements of different muscles are generated by changes of rotational and screw symmetry. The particular packing of rods discussed by Squire¹⁴ as a model for all myosin filaments is more compact than the present structure: groups of rods do not twist round each other in subfilaments near the surface, but instead form radial sheets which more nearly fill the filament centre. The present X-ray data require a backbone organised so that there is a marked circumferential repeat of 40 Å rather than the less 'contrasty' surface appearance of the model favoured by Squire¹⁴.

There are two reasons why X-ray patterns from crustacean muscles are especially informative about backbone structure. First, the pronounced twisting of the subfilaments round the filament means that a key diagnostic layer line can be observed, separated from the equator of the X-ray pattern. Second, the distinct but related filament structures in the various crustacean muscles indicate the conserved and the variable aspects of the molecular packing. Corresponding data from other animals will establish how far myosin backbones in muscles outside this restricted class can be described by the present model. X-ray data on cross-bridge arrays in some invertebrate muscles apart from crustaceans (such as *Lethocerus* flight muscle¹³ and *Limulus* muscle¹⁵) can be interpreted in terms of a simple helical surface lattice: backbone models for these filaments must again be such that myosin molecules occupy equivalent positions. In fibrillar insect flight muscles, for example, the 385-Å myosin helical repeat¹¹ may result from further change of screw symmetry in a filament of the crustacean type, as a reflection with the axial spacing of roughly 1,150 Å, as expected for diffraction from subfilaments (see legend to Fig. 2), is observed at 40 Å radius (my unpublished results on bee and fly muscles, and ref. 16). The flight muscles of some dragonflies (Odonata) are non-fibrillar, and support this extension of the crustacean model to include insect filaments. Their myosin symmetry differs from that in the fibrillar muscles: the helical repeat is about 350 Å, and the subfilament diffraction is very like that of slow crustacean muscles. Application of the subfilament model to insect as well as to crustacean filaments does not demand the same rotational symmetry in both cases, but does leave the puzzle that, just as argued above for crustaceans, the 150–200-Å diameter of insect filaments observed by electron microscopy¹⁷ could accommodate 12 subfilaments, and thus 4 myosin molecules per 145 Å of filament length, while other evidence (see ref. 18) suggests 6 myosins per 145 Å for insect muscle.

Vertebrate and other filaments

In some other muscles, such as vertebrate striated muscles⁹ and the striated adductor of the scallop^{10,19}, X-ray evidence suggests that the cross-bridge array departs from equivalence in that adjacent 145-Å levels are not identical (possibly because of non-equivalent packing in the backbone or a perturbation due, for example, to proteins other than myosin). Here I consider non-equivalence only in the context of the present subfilament model, retaining the 435-Å repeat within the subfilament and the 145-Å stagger between subfilaments as conserved features. Filaments built in this way provide equivalent environments for all myosin molecules only when each subfilament, at every level at which cross-bridges emerge from it, appears at the filament surface in an identical way. Disposition of the subfilaments to form tubular filaments in crustaceans fulfills this requirement in a simple manner (which, however, leaves a large core). But strict equivalence is not necessarily a significant property for the cross-bridge array: the departures detected by X-ray diffraction may be rather small, and may leave all myosin molecules functionally equivalent in their ability to interact with actin. More compact structures would result from an orderly collapse of the tubular form (with loss of perfect helical symmetry, as cross-bridges from more central subfilaments would necessarily

adopt slightly different resting configurations). Collapse is plausible only when the subfilaments run almost parallel to the filament axis (as inner and outer subfilaments otherwise acquire different axial repeats), or when the filament is very short, but could in either of these cases occur with little change in the pattern of intermolecular connections. Vertebrate skeletal myosin filaments might exemplify this more radical transformation of the crustacean structure, as their screw symmetry is such that the subfilaments would run parallel to the axis (see legend to Fig. 2). Collapse could give solid polygonal filaments as observed for frog and fish muscles^{20,21}. The consequent non-equivalence would be additional to that resulting because C-protein is not located according to the 143-Å repeat of myosin²². Such a model for vertebrate filaments remains entirely speculative until direct information about their backbone structure is obtained, but meanwhile illustrates one natural way for more specialised myosin structures to be generated from a simple, versatile and widespread design.

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letters

Are there organic grains in the interstellar medium?

THE possibility that organic compounds exist in or on interstellar grains has received much attention recently. It has been suggested that grains consist of polysaccharides¹, carbonaceous compounds² or aggregates of a variety of organic compounds³. While it is easy to visualise how some of these materials could form in the interstellar medium (for example, by the random accretion of C, N, O and H) it is not certain that appreciable quantities of such grains actually exist. With the availability of high quality infrared (IR) spectra of a variety of sources we show here how to determine the fraction of interstellar carbon bound in complex molecules of this sort.

Virtually all compounds containing C and H possess an IR absorption band due to the excitation of the C–H stretching vibration between 3.3 and 3.4 μm . This band usually has a width of ≈ 0.05 – $0.2 \mu\text{m}$ in condensed phases. As noted by Knacke² this band will be present even in polymeric or tarry materials as found, for example, in carbonaceous chondrites. The persistence of a band at 3.3–3.4 μm in all organic systems implies that observations of the spectrum of interstellar dust in this region can be used in a determination of the abundance of organic solids in interstellar dust.

Figure 1 shows a plot of $\tau(3.3\text{--}3.4 \mu\text{m})$ against A_v , the visual extinction in magnitudes, for a variety of organic compounds representative of several chemical classes. These optical depths at 3.3–3.4 μm would be obtained if all interstellar C were obtained in these molecules. They are calculated on the basis of an extinction $A_v = 1.6 \text{ mag per kpc H atom cm}^{-3}$ using the normal cosmic abundance of carbon⁴. Data on the strength of the 3.3–3.4 μm band was obtained from standard sources^{5,6}. Cross-sections vary from a low of $3.4 \times 10^2 \text{ cm}^2 \text{ g}^{-1}$ for C_6H_6 to $8.4 \times 10^3 \text{ cm}^2 \text{ g}^{-1}$ for $\text{C}_{42}\text{H}_{88}$ (2,2,4,15,17,17-hexamethyl-7,12-di(3,3,5-trimethyl hexyl)octadecane). It can be seen that the largest values of τ are produced by alkanes. The cyclic ether, propylene oxide also produces strong absorption at 3.3–3.4 μm . Aromatic compounds such as C_6H_6 are relatively weak absorbers although substitution with any CH bearing group (such as *p*-xylene) greatly increases the 3.3–3.4 μm band strength. Polymers such as polyethylene and polyvinyl alcohol are strong absorbers.

The corresponding relationship between equivalent width, W , in μm and A_v is

$$\frac{W}{A_v} = 2.9 \times 10^{-2} \frac{I}{n_c} \quad (1)$$

where I = integrated 3.3–3.4 μm band intensity in units of $10^4 \text{ l cm}^{-2} \text{ mol}^{-1}$ and n_c = number of carbon atoms in each

absorbing molecule. This equation is obtained from Wexler's expression⁶ for integrated intensity in these units, $I = \Delta\nu \log_{10}(\mathcal{I}_0/\mathcal{I})/(10^4 CL)$ where $\Delta\nu$ is FWHM if cm^{-1} , $\mathcal{I}/\mathcal{I}_0 = e^{-\tau}$ is the intensity ratio in the line, C is the concentration in moles per 10^4 litre, and L is the path length in cm. Most bands have intensities, in these units, in the range 0.01–1, (ref. 6). For small τ , $W = \tau\Delta\lambda$, and so rearranging, with some attention to units, gives $W = N\lambda^2 I/2.62 \times 10^{20}$. W and λ are in μm , and N is the column density (cm^{-2}) of absorbing molecules. Thus $N = [C]L/n_c$ where $[C]$ is the total available carbon density (cm^{-3}). With extinction A_v of 1.6 mag kpc^{-1} per H-atom, then $A_v = 1.6[H]L/3 \times 10^{21}$. Using the cosmic abundance of carbon, the ratio of W to A_v gives equation (1). Since $I = n_c G$ where G = integrated intensity per structural unit (CH , CH_2 or CH_3),

$$W = 2.9 \times 10^{-2} A_v G \quad (2)$$

G is quite different for molecules of different chemical classes but is relatively constant for molecules of the same class⁶. Figure 1 shows data for a selection of molecules that are representative of the major chemical classes. Obviously under interstellar conditions a variety of molecules would be expected and so an average would have to be taken to determine the total expected 3.3–3.4 μm optical depth.

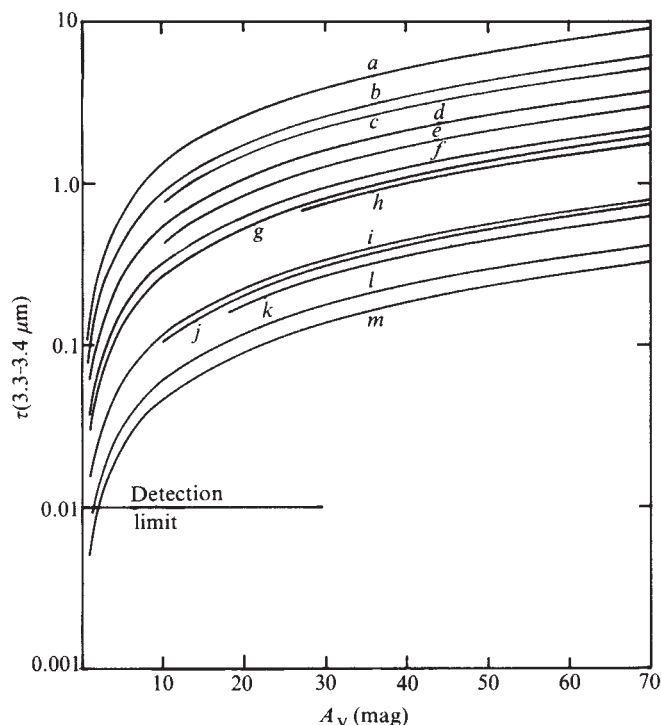


Fig. 1 Predicted optical depth at 3.3–3.4 μm , τ due to organic compounds on grains plotted against visual extinction A_v in magnitudes. A detection limit provided by recent observations⁷ is shown at $\tau = 0.01$. Failure to detect the presence of a 3.3–3.4 μm band with this amplitude in an object with $A_v = 70 \text{ mag}$ would imply that $<0.2\%$ of interstellar carbon is present as alkanes: *a*, $\text{C}_{42}\text{H}_{88}$; *b*, *n*-hexane; *c*, propylene oxide; *d*, 1-pentanol; *e*, *n*-butyl ethyl ether; *f*, polyethylene; *g*, acetone; *h*, poly-vinyl alcohol; *i*, *p*-xylene; *j*, 1–3 butadiene; *k*, pyridine; *l*, 3-octyne; *m*, benzene.

A line in Fig. 1 indicates the current detection limit for spectral features in the 2.9–3.4 μm region as obtained, for example, by McMillan⁷. Spectroscopic data of high quality obtained by Merrill *et al.*⁸ for a variety of highly obscured objects shows no evidence for detectable 3.3–3.4 μm absorption even in objects with $A_v \geq 50 \text{ mag}$. It is also significant that no absorption appears at 3.3–3.4 μm even in objects with well developed 3.08 μm ice bands. These observations, together with the curves given in the Fig. 1 suggest that the limits shown in Table 1 can be placed on the abundance of organic compounds on dust in the interstellar medium.

Table 1 Abundance limits of various chemicals in the interstellar medium

Chemical class	Abundance limit
Alkanes	$<1\%$ of C
Cyclic ethers	$<1\%$
Non-cyclic ethers	$<2\%$
Alcohols	$<2\%$
Polymers	$<2\%$
Ketones	$<2\%$
Alkenes	$<10\%$
Alkynes	$<10\%$
Aromatics	$<10\%$

These limits are expressed in terms of the percentage of carbon that could be present in molecules of each class without producing $\tau(3.3\text{--}3.4) > 0.05$ for $A_v = 70 \text{ mag}$. As all molecules absorb at the same wavelength it is not possible to sum abundance limits in Table 1 to permit the dispersal of larger amounts of carbon in organic compounds. Thus if 1% of C were in alkanes, $\tau = 0.05$ would be produced by these molecules alone implying that the abundances of all other types of organic molecule would be much less than those given in Table 1.

We conclude that no spectroscopic evidence exists to support the contention that much of interstellar dust consists of organic materials. While the presence of trace quantities of organic compounds on grains inside very dense clouds cannot be excluded by available data, the absence of any observation of a 3.3–3.4 μm absorption band even in objects with $A_v \approx 50 \text{ mag}$ strongly suggests that organic grains constitute at most a minor component of interstellar dust and probably have no observable effect on visual extinction. Certainly the dust that produces the visual extinction in highly obscured sources cannot be composed of organic material. For example, in an object with $A_v = 50 \text{ mag}$ the column density of dust in the form of particles of radius 10^{-5} cm with scattering efficiency = 1, is $(N L)_{\text{dust}} = 1.6 \times 10^{11} \text{ cm}^{-2}$. For a grain density of 1 g cm^{-3} the mass of dust per cm^2 is $6.4 \times 10^{-4} \text{ g cm}^{-2}$. Then $\tau(3.3\text{--}3.4) \text{ C}_6\text{H}_6 = 0.22$ and $\tau(3.3\text{--}3.4) \text{ C}_{42}\text{H}_{88} = 5.4$ in this object. Optical depths in this range would certainly be detectable.

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Magnetic buoyancy in the Sun

THE concept of 'magnetic buoyancy' was introduced¹ as a possible mechanism by which magnetic fields from within the Sun might come to the surface and contribute to its magnetic activity. We discuss this concept here and consider its effect on solar dynamics.

An isolated flux tube of field strength $\mathbf{B}$ in a gaseous atmosphere of magnetic permeability μ has a lower thermodynamic pressure p than that of its surroundings, because the total

pressure $p + \frac{1}{2}\mu^{-1}\mathbf{B}^2$ must be the same inside and outside the tube. If the temperatures inside and out are sufficiently close it will then be lighter than its surroundings and will tend to rise by this magnetically-induced buoyancy.

Recently^{2,3} it has been argued that this mechanism actually works rather too well for comfort, bringing 100-G flux tubes from all but the bottom quarter of the solar convection zone (depth $\sim 200,000$ km) to the surface in a good deal less than the time needed for the generation of these fields by the hydrodynamic dynamo process⁴, which is 10 yr, that is, comparable with the time scale of the solar cycle. As far as the origin of these thin flux tubes is concerned, it has been pointed out that a large-scale azimuthal field which decreases with height is unstable³, again by the mechanism of magnetic buoyancy, and would break up in the required way within a time of order H/V , where H is the scale height for the density ρ and

$$V \equiv B/(\mu\rho)^{1/2} \quad (1)$$

is the Alfvén speed. This 'break-up' time is also much less than 10 yr except at the very bottom of the convection zone. The conclusion of this argument is that the dynamo must function at the very bottom of the zone with a field strength of ~ 100 G; such a field is barely enough to account for the total flux emerging from the Sun in the course of one solar cycle³.

As flux tubes rise they encounter lower background pressures and if they are horizontal they expand in cross-section. Their magnetic field therefore drops, and it has been argued⁵ that the concomitant reduction in their magnetic buoyancy can substantially increase the time they take to reach the solar surface. The matter is complicated, however, by the fact that by bending, so that some portions are higher than others, the 'crests' can largely retain their magnetic buoyancy by off-loading¹ some of the gas down the arms of the tube to the 'troughs', which proceed upwards rather more slowly as a result. The initial instability mechanism certainly uses this process, so that the most rapidly growing disturbances have non-zero wavelength (in fact, comparable with the magnetic field scale-height) along field lines^{6,7}, and it is natural to expect this bending of the field lines to persist even when the amplitude of the disturbance becomes large.

Further bending of the tube, of a different kind, will result from the action of the Coriolis force (due to the Sun's angular velocity Ω) on the material sliding down from the crests to the troughs; this imparts an anticyclonic twist ψ to the crests, which as a result do not break the surface quite along a line of latitude. This is thought to explain the fact that the 'leading' sunspot of a bipolar pair is normally closer to the Equator than the 'following' spot⁸. The observations demand that typically $\psi \sim 10^\circ$.

Recent studies of instability by magnetic buoyancy in a rotating system⁸⁻¹⁰, however, suggest the solar rotation exerts a much stronger influence than this on the entire mechanism: the dimensionless parameter

$$S \equiv \frac{\Omega H}{V} \quad (2)$$

is large throughout most of the convection zone for an azimuthal field of 100 G, ranging from ~ 20 at a depth of 30,000 km to ~ 500 at the bottom of the zone ($\sim 200,000$ km depth). It turns out that magnetic buoyancy instability does not then develop on the Alfvén time scale H/V at all, but on the much longer time scale

$$T \sim \frac{4\Omega H^2}{V^2} \quad (3)$$

This is ~ 10 yr at 30,000 km depth, again for a field of 100 G, but far exceeds the time scale of the solar cycle at greater depth. Further, if the effects of turbulent diffusion are taken into account instability does not take place at all unless

$$\mathcal{C} \equiv \frac{V^2}{2\Omega\eta} \quad (4)$$

is greater than about unity. With turbulent diffusivities η of the

kind usually envisaged^{11,12}, that is 10^8 – 10^9 m² s⁻¹, $\mathcal{C}$ is $\sim 10^{-3}$ or so throughout much of the zone.

Suppose, therefore, that the azimuthal field were much larger, say at least 1,000 G. The parameter $\mathcal{C}$ might just about exceed unity (η is somewhat uncertain¹²), while the time scale T ranges from a few weeks at depths of 30,000 km to ~ 10 yr at 100,000 km. In the bottom half of the convection zone we would not expect even a 1,000-G field to break up by magnetic buoyancy instability within the time scale of the solar cycle.

The most rapidly-growing mode has a wavelength along field lines of order H , as in the absence of rotation, and the original field again breaks up into isolated flux tubes with 'crests and troughs'. What happens to these tubes when they have floated up and distorted a significant amount is beyond the scope of the linear instability theory, but it seems natural to suppose that the rate of rise will then be comparable with the actual gas velocities $\mathbf{u}$ involved, and that the time scale will be comparable with that for the instability. Then the rate of rise and $\mathbf{u}$ will be of the order $V^2/\Omega H$, which is much smaller than the Alfvén speed, and implies that the Coriolis forces (per unit mass) $2\rho\Omega\mathbf{u}$ and the Lorentz forces $\mu^{-1}(\nabla\wedge\mathbf{B})\wedge\mathbf{B}$ will be of comparable magnitude. This avoids a contradiction that arises if one assumes that rotation plays only a rather minor role and that the rate of rise and $\mathbf{u}$ are of order V (see above): the ratio of Coriolis forces to Lorentz forces is then $\Omega H/V$ and very large (for a 100 G field), indicating that rotation must, in reality, have had a crucial effect on the motion of the tube.

Consideration of magnetic buoyancy may place restrictions on solar dynamics which are rather different to those which have recently been suggested, because the rotation of the Sun has a very strong influence on the mechanism. Fields of 1,000 G might be retained in the middle of the zone for 10 yr or so; deeper down even stronger fields could be retained. If magnetic buoyancy is responsible for bringing to the surface the fields that are observed, their origin must be strong ($\geq 1,000$ G) azimuthal fields in the top half of the convection zone. Such fields would make it easier to account for the total flux emerging from the Sun in the course of a solar cycle³, and would also give the rising flux tubes more resistance to Coriolis twisting, which together with a shorter travel distance to the surface (compared with the full depth of the convection zone) would help keep ψ at a relatively small value.

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Possible use of ⁴¹Ca for radioactive dating

A LARGE number of cosmogenic isotopes have been suggested for use in radiometric dating¹. One which has not received much attention is ⁴¹Ca (half life $t_{1/2} = 1.3 \times 10^5$ yr (ref. 2)). This is probably due to its small expected concentration in natural samples and the difficulty in detecting its pure electron capture

decay. On the other hand, the importance of calcium in many geochemical and biochemical cycles ensures that any method of dating this element would have widespread application. Recent important developments in the detection of radioisotopes using accelerators have motivated us to look at this question. We show here that use of ^{41}Ca as a dating tool may indeed be feasible.

Most proposed cosmogenic dating nuclides are formed by the interaction of cosmic-ray primary and secondary particles with the Earth's atmosphere. The only significant components of the atmosphere which can give rise to ^{41}Ca are krypton and, with low energy α particles, argon. As the abundance of Kr in the atmosphere is only $\sim 10^{-4}\%$, and the effect of α particles small, production by this means is also small. Calcium is, however, a fairly abundant element in the Earth's crust. Here, the reaction $^{40}\text{Ca} (n, \gamma) ^{41}\text{Ca}$ can take place with slow neutrons. At the Earth's surface such a process is favoured both because of a fairly large cross-section ($\sigma = 440$ mb for thermal neutrons), and because the downward moving neutrons in the atmosphere are rapidly thermalised by the ground, giving rise to a significantly enhanced slow neutron flux at the air-surface interface³.

The concentration of ^{41}Ca after exposure to a flux (f) of neutrons for a time (t) is given by

$$^{41}\text{Ca}/^{40}\text{Ca} = \frac{ft t_{1/2}}{0.693} (1 - \exp(-0.693t/t_{1/2}))$$

Using a value of $3 \times 10^{-3} \text{ n cm}^{-2} \text{ s}^{-1}$ for the thermal flux³, and $t \gg t_{1/2}$, we get a 'saturation' value of $^{41}\text{Ca}/^{40}\text{Ca} \sim 8 \times 10^{-15}$. This corresponds to an activity of $\sim 1 \times 10^{-3}$ disintegrations per minute per gramme of Ca—about 10^4 times smaller than for contemporary ^{14}C . In addition, the decay of ^{41}Ca takes place by orbital electron capture directly to the ground state of ^{41}K . Therefore, the only radiation given off is the low energy (3.3 keV) X ray from the ^{41}K . It is these factors which have discouraged consideration of this isotope as a dating possibility.

Radioisotope detection with accelerators⁴ has the advantage of high sensitivity, independent of the mode of decay. The technique has already been successfully applied⁴⁻⁸ to measurement of ^3H , ^{14}C , ^{10}Be and ^{36}Cl , and shows great promise for some other long-lived isotopes (several variants of this technique are described in ref. 9). To measure a $^{41}\text{Ca}/^{40}\text{Ca}$ ratio of $\leq 10^{-14}$, a machine capable of producing a ^{40}Ca beam of $\geq 10^{13}$ particles per second would be desirable. As each gramme of Ca having the above ratio contains only $\sim 10^8$ atoms of ^{41}Ca , we would also like to have an overall acceleration efficiency of $\geq 10^{-5}$. For reasons given below, it might be necessary to accelerate the ^{41}Ca ions to an energy of ≥ 300 MeV. There is at least one accelerator in which Ca beams meet the above requirements¹⁰. Several superconducting or two-stage accelerators proposed or currently under construction should also have such a capability. Also, by including a 'pre-enrichment' step (for example, using an isotope separator), the above intensity requirement could be reduced significantly.

The main interference in such a measurement is expected to come from saturation of the detecting element with a large flux of natural ^{41}K . In this case the technique of absorbing the interfering ion⁴ cannot be used because the range of the ^{41}K is greater than for ^{41}Ca . It may be possible to make a sufficiently good chemical separation of the sample to avoid this difficulty. However, an even more elegant technique would be to use the fact that ^{41}K can never have an atomic charge of +20. Thus, by accelerating the ^{41}Ca ions to sufficient energy (≥ 300 MeV) for a significant fraction of them to be fully stripped, they can be magnetically separated from the ^{41}K . We have recently confirmed the feasibility of this technique by separating 200-MeV beams of ^{26}Al and ^{26}Mg at the ALICE accelerator at Orsay (unpublished work). Such a procedure would also be applicable to other isotopes, such as ^{36}Cl , ^{53}Mn and ^{59}Ni , which have nuclear charges greater than an interfering isobar.

To be of optimum use as a dating tool, the production and decay of the radionuclide must be clearly separated in time.

With species formed in the atmosphere, this separation is quite natural. For a radionuclide formed in the lithosphere, such a separation means that the material being considered must be removed from (or exposed to) the source of the irradiation flux in a time which is short compared with the relevant half-life. For nuclear active bombarding particles such as neutrons, the shielding required to reduce production to negligible levels is of the order of 500 g cm^{-2} .

Some of the interesting possibilities of using isotopes formed in this way have been discussed by Davis and Schaeffer¹¹ in connection with ^{36}Cl . For example, one can determine when presently exposed samples were brought to the surface (such as by volcanoes, glaciers, or changing river patterns).

In the case of Ca, a potentially interesting application is the dating of CaCO_3 in sediments. Here the 'age' measured would be the time since the ^{41}Ca was removed from its equilibrium concentration in the overlying water. The most promising environments for such an application are bodies of water (such as lakes) where the Ca residence time is short compared with the ^{41}Ca half-life. In the oceans, the estimated residence time is $\sim 10^6$ yr (ref. 12). As this is about seven times the ^{41}Ca half-life, the equilibrium ratio of $^{41}\text{Ca}/^{40}\text{Ca}$ in the oceans is expected to be about two orders of magnitude less than the injection ratio. A $^{41}\text{Ca}/^{40}\text{Ca}$ ratio of 10^{-16} or smaller would almost certainly necessitate a pre-enrichment step before measurement with an accelerator. In addition, at such low concentrations one must begin to worry about other rare production modes, such as those induced by muon capture or natural radioactivity. However, even here a comparison of the injection ratio of $^{41}\text{Ca}/^{40}\text{Ca}$ with that found in various parts of the oceans (or sediments) might be useful for studying ocean circulation patterns.

The most exciting application of ^{41}Ca is that it might be used for dating animal (including hominid) remains in the interval of $\sim 5 \times 10^4$ – 10^6 yr: the bones of most vertebrates consist of calcium phosphate. Most of this Ca comes, directly or indirectly, from plants which have absorbed it from near surface water. The mean rate of erosion on the Earth's surface is estimated to be $\sim 5 \text{ cm per } 10^3 \text{ yr}$ (ref. 13). Thus, on average, material will rest within 1 m of the surface for $\sim 20,000$ yr. Of course, the actual values vary considerably, depending on the location, climate, type of rock and so on (for example, in low relief tropical regions, which may be among the most interesting for this application, the estimated surface residence time can approach 10^6 yr (ref. 13)). In addition, being readily soluble, there can be exchange between surface Ca and that dissolved from deeper sources. At present we find it difficult to evaluate the overall effect of these factors. Ultimately they can probably only be determined by experiment. However, ignoring the effects of exchange, we estimate that surface water would typically have a $^{41}\text{Ca}/^{40}\text{Ca}$ ratio of $\sim 10^{-15}$. In such a circumstance, ^{41}Ca would offer a possibility for dating in an analogous way to ^{14}C . One could take advantage of the fact that the living sites for early hominids were often grottos having the prerequisite shielding to stop production of ^{41}Ca . Thus the $^{41}\text{Ca}/^{40}\text{Ca}$ ratio in fossil remains in such sites, compared with the initial ratio, would be a measure of their age. Even if, as is likely, the initial $^{41}\text{Ca}/^{40}\text{Ca}$ ratio were not universally constant, it might be possible to 'calibrate' the technique at various epochs and locations, using dates obtained by other methods.

Although it will take a careful evaluation, and eventually experimental verification of the assumptions made here, we believe that radioactive dating using ^{41}Ca may turn out to be a very important procedure. We are optimistic enough to be currently working on an experimental test of these ideas.

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Strontium isotope evidence for petrogenesis of Central American andesites

THE active volcanic belts of Mexico and of Central America can be considered in two groups (Fig. 1). The Mexican volcanic belt (MVB) forms an irregular line trending away from the Middle America Trench, running from Ceboruco and Colima in the west to San Martin on the Caribbean coast¹. In contrast to the MBV, the active Central American volcanic belt runs some 1,200 km from southern Mexico (Tacaná) and Guatemala to Costa Rica, broadly parallel with the Middle America Trench¹. With the exception of the alkaline volcano San Martin², these are all calc-alkaline andesite volcanoes¹. Between Mexico and El Salvador, the volcanic chain is built on a crust which includes Precambrian and Lower Palaeozoic crystalline rocks^{3,4}. South-east of El Salvador, in Nicaragua and Costa Rica the crust is composed mainly of Cainozoic volcanic and sedimentary rocks resting on Cretaceous ultramafic complexes^{3–6}. This change in basement character does not seem to be reflected in the composition of volcanic rocks⁷. Here we report new Sr isotope data for Cainozoic volcanic rocks from Costa Rica, and by comparing these with earlier published data we conclude that all of these volcanic rocks have their origin in the mantle and that a sialic contribution to their origin is insignificant.

Sr isotope data for Cainozoic volcanic rocks from Central

America have been published for most of the area shown in Fig. 1. These include data for Mexico^{8,9}, Guatemala^{10,11}, El Salvador and Nicaragua¹⁰, and Costa Rica¹². In addition to the volcanic rocks, Pushkar has reported Sr isotope data for pre-Cainozoic basement rocks from Guatemala and Honduras¹⁰. For Costa Rica, the only reported Sr isotope data are seven analyses determined by Montigny *et al.*¹². These include one and four samples respectively from the active volcanoes Irazú and Poás.

We have determined Sr isotope data for 10 Recent lavas and juvenile blocks from the active volcanoes Arenal (four), Poás (four) and Irazú (two). The lavas from Poás are Pleistocene–Recent, whereas those from Arenal were erupted during 1978, and the blocks from Irazú were erupted during 1963–64. Sr isotope ratios were determined on a VG-Micromass 30 mass spectrometer using chemical and mass spectrometric techniques described elsewhere¹³. Measurements of the Eimer and Amend standard SrCO₃ carried out during this work yield an overall mean of 0.70812 ± 1 . Rb/Sr isotope ratios were determined by a precise X-ray fluorescence technique¹⁴. The Sr isotope ratios and related analytical data for the Costa Rican lavas are presented in Table 1. Because the analysed samples are all geologically young (<1 Myr, but most are <20 yr), the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios presented in Table 1 are taken as initial ratios. These data are shown as a plot of $^{87}\text{Sr}/^{86}\text{Sr}$ against $^{87}\text{Rb}/^{86}\text{Sr}$ in Fig. 2, which also shows fields of some previously determined Sr isotope data.

Table 1 shows that Sr isotope compositions of Costa Rican lavas from Arenal and Poás show slight but significant internal variation within the range 0.7036–0.7042. The samples from Irazú have $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.7036–0.7037. Although there is considerable variation in Rb/Sr (similar to that shown by Mexican lavas) there is no correlation of $^{87}\text{Sr}/^{86}\text{Sr}$ with $^{87}\text{Rb}/^{86}\text{Sr}$ either for individual sets of data or for the data as a whole. The relatively imprecise Sr isotope ratios of 0.7037–0.7055 reported for Costa Rican lavas by Montigny *et al.*¹² are within the range of Sr isotope data we have reported for the Mexican andesite volcanoes of Nevado de Colima, Colima, and Ceboruco, and the alkali basalt volcano San Martin⁹. Sr isotope values (also determined in the Oxford Isotope Laboratory) for lavas from the Guatemalan volcanoes Santa Maria and Santiaguito are 0.70396 ± 6 (ref. 11) and hence indistinguishable from the values reported above. Earlier Sr isotope ratios determined for lavas from Guatemala, El Salvador and Nicaragua by Pushkar¹⁰ fall within the range 0.7031–0.7049 (13 samples), but are mostly within the range 0.7037–0.7044 (11 samples; see Fig. 2). In contrast, Sr isotope values for pre-Cainozoic metamorphic and igneous rocks from Guatemala, Honduras and Nicaragua are largely within the range 0.71–0.75 (ref. 10).

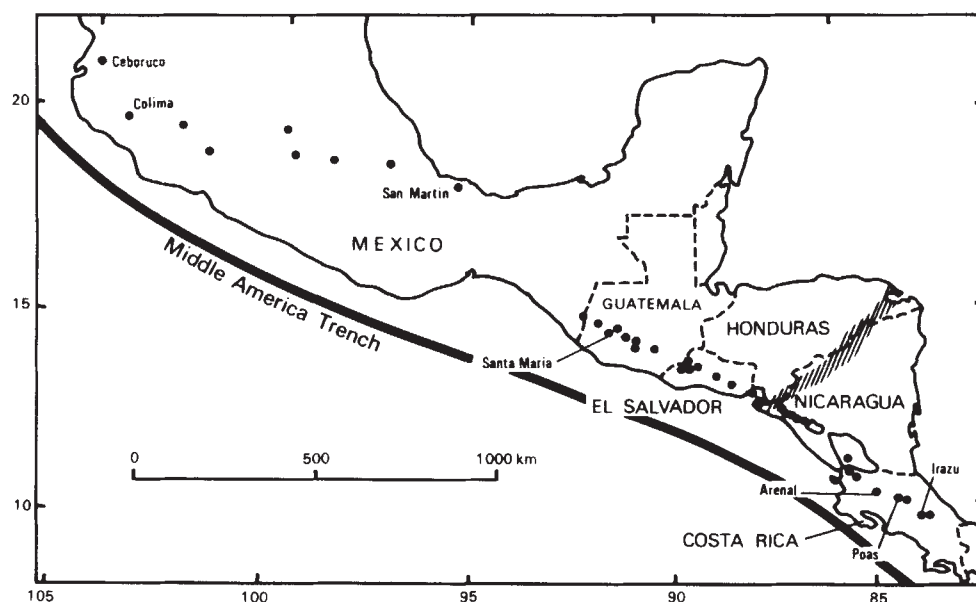


Fig. 1 Volcanoes and plate tectonics of Mexico and Central America. ●, Recently active volcanoes. The shaded area between Honduras and Nicaragua separates an area with older pre-Mesozoic crust to the north-west from an area with continental crust of upper Mesozoic–Cainozoic crust to the south-east. See text for further discussion.

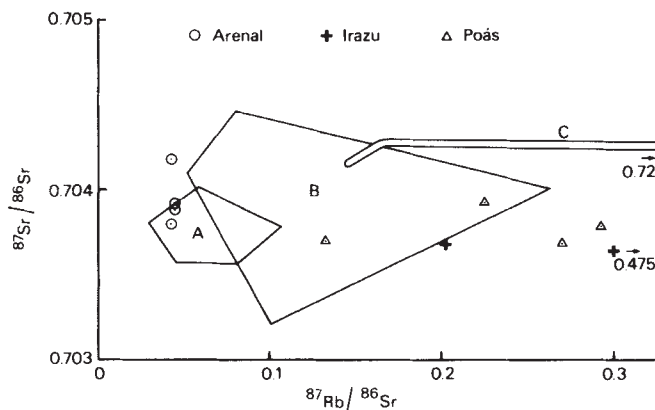


Fig. 2 Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ against $^{87}\text{Rb}/^{86}\text{Sr}$ for volcanic rocks from Mexico and Costa Rica. The outlined fields A–C are for volcanic rocks from Mexico (refs 8 and 9). Field A is for lavas from San Martín, B is for lavas from the Colima and Toluca areas and C is for lavas from Ceboruco (see ref. 9, Fig. 2).

The above Sr isotope ratios for volcanic rocks of Mexico and Central America are all within the range reported for lavas from volcanic arcs where sialic crust is absent^{10,15–17} and show no significant difference between the MVB and north-west Central America (pre-Cainozoic sialic crust) and south-east Central America (largely Cainozoic sialic crust). The low Sr isotope ratios for Mexican and Central American andesites, commonly within the range 0.7035–0.7040, suggest that sialic bulk contribution to their origin is insignificant.

The Sr isotope data provide clues to the origin and subsequent evolution of andesitic magma. First, the new results confirm that andesites from many oceanic and continental provinces are derived from a source region with $^{87}\text{Sr}/^{86}\text{Sr}$ ratios within a relatively narrow range of ~0.7035–0.7045 (see refs 9, 18, 19). This range is slightly but significantly higher than the source region of ocean floor basalts (0.702–0.703; ref. 18), and the data could be interpreted in terms of a model, developed from Sr and Nd isotope data, in which andesites are produced by partial melting of mantle wedge which has been enriched in ^{87}Sr as a result of fluids driven off the descending oceanic slab^{24,25}. However, the Sr isotope compositions of the andesites lie within the range reported for oceanic and continental alkali basalts^{9,18,20–23}, and derivation from such a mantle source cannot

be excluded²⁶. Hence variation in the Sr isotope composition might result from small-scale mantle inhomogeneities. This cannot be evaluated without further Sr and Nd isotope studies, but data reviewed below suggest that some of the small-scale Sr isotope variations might result from processes occurring within the crust rather than the mantle.

The ^{238}U – ^{230}Th radioactive disequilibrium method has been applied to Costa Rican lavas by Allègre and Condomines²⁷. These workers analysed rock and mineral samples from Irazú (six samples, including a 'volcanic bomb' from the 1964 eruption, Poás (two samples) and Arenal (one sample). For the Irazú samples, ^{238}U – ^{230}Th mineral isochrons indicate crystallisation of the lavas at times between 1.1×10^5 yr and the present day (equivalent to 1964). By considering the evolution of the initial $^{230}\text{Th}/^{232}\text{Th}$ ratio with time, Allègre and Condomines argue that the Irazú lavas evolved from a magma chamber established at 1.4×10^5 yr BP, with $^{230}\text{Th}/^{232}\text{Th} = 0.81$. Assuming a similar initial $^{230}\text{Th}/^{232}\text{Th}$ and source U/Th ratio, the samples from Arenal and Poás must have evolved from independent magmatic systems established respectively at 3.5×10^4 yr and 5×10^4 yr BP. These data may be interpreted as indicating the presence of a magma chamber below each of the three Costa Rican volcanoes. It seems plausible, therefore, that small variations in Sr isotope composition, both within and between volcanoes, are the result of processes (such as selective contamination) which take place within the magma chamber.

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Table 1 Analyses of Costa Rican lavas

Specimen No.	Rb* (p.p.m.)	Sr* (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}^\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}^\ddagger$
Arenal				
AR1	10.5	697	0.043	0.70417 ± 8
AR2	11.2	709	0.046	0.70392 ± 8
AR3	10.9	702	0.046	0.70389 ± 8
AR4	10.9	709	0.043	0.70380 ± 5
Poás				
P5	39.9	509	0.226	0.70392 ± 7
P10	48.7	486	0.289	0.70378 ± 7
P50	48.5	524	0.269	0.70365 ± 8
P52	26.5	580	0.133	0.70371 ± 7
Irazú				
IR1	54.4	780	0.203	0.70369 ± 7
IR2	105	643	0.475	0.70364 ± 6

* Determined by X-ray fluorescence. Mass absorption coefficients estimated from Compton scattering, concentration data about $\pm 5\%$.

† Average precision $\pm 1\%$ (for X-ray fluorescence techniques, see ref. 14).

‡ Within-run precision quoted at 2σ error.

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Calibration of Grenvillian palaeopoles by $^{40}\text{Ar}/^{39}\text{Ar}$ dating

PALAEOPOLES from the exposed Grenville Province of the Canadian Shield are displaced from those of the remaining shield. It has been proposed that these poles record either a Precambrian plate collision $\sim 1,000$ Myr (refs 1–3) or manifest a large rotation and translation of the entire extant North American crust^{4–6}. Geological evidence in support of the collisional hypothesis is largely lacking; for example, an acceptable suture zone has not been located. The critical evidence for a choice between the two-plate and one-plate tectonic hypotheses must, therefore, come from dating these palaeopoles because if the magnetisations were acquired contemporaneously with those of the Logan Loop ($\sim 1,000$ – $1,200$ Myr), then the two-plate or collisional hypothesis is probably correct. We present here an empirically derived cooling curve for the Haliburton basic intrusions within the Grenville Province. These intrusions are located in a region of high-grade metamorphism, upper amphibolite to granulite facies. Their emplacement times are not known, but in the Haliburton Highlands cooling from the culmination of regional metamorphism, not contemporaneous throughout the Grenville Province, began $\sim 1,050$ Myr ago⁷. This is the first cooling curve for an orogen to be derived solely from isotopic data; that is, the first for which both temperature and time have been determined from the same samples. Using this as a calibration curve, we show here that the thermal remanent magnetisation (TRM) components from the Haliburton basic intrusions were acquired between ~ 980 and 820 Myr ago during slow cooling and that, consequently, the two-plate hypothesis is improbable.

With the rapid extension in recent years of palaeomagnetism to Precambrian rocks in orogenic belts and the subsequent discovery of multicomponent natural remanent magnetisations (NRM) (see ref. 8 for references), it has become clear that the acquisition times for TRM components are not always directly defined by an isotopic date. In slow cooling conditions, the Rb–Sr whole-rock and K–Ar mineral clocks will record different times. Hence, the TRM acquisition times will be indeterminate. Furthermore, for a history of successive reheating episodes, rather than one of slow cooling, the conventional K–Ar and Rb–Sr dating techniques often lack the requisite discrimination so that partially reset dates will be confused with totally reset dates.

To overcome these limitations, we have applied detailed (15-step) incremental heating, $^{40}\text{Ar}/^{39}\text{Ar}$ experiments to a variety of minerals, including some types traditionally shunned in K–Ar dating. These minerals were chosen from the same samples studied palaeomagnetically³ from the Bark Lake and Dudmon dioritic bodies and the Glamorgan gabbro-anorthosite complex in southern Ontario. Using the $^{40}\text{Ar}/^{39}\text{Ar}$ method as a diffusion experiment, we have calculated Ar closure temperatures for each mineral with Dodson's formula⁹, as first suggested by Buchan *et al.*⁸. The required diffusion coefficients were calculated by the method of Fechtig and Kalbitzer¹⁰. These closure temperatures are insensitive to uncertainties in the assumed cooling rate⁹ and so the formula can most profitably be applied to Precambrian orogenic belts where a slow cooling following uplift and erosion is likely to have occurred. By thus establishing a cooling curve from isotopic studies, we can directly date TRMs using their empirical blocking temperatures.

In detailed palaeomagnetic studies^{3,8}, the Haliburton intrusions have yielded three NRM components, two of which are probably partial TRMs (PTRM) and sometimes occur within the same specimen. The component with the highest laboratory blocking temperature gives a palaeopole, Hb₁, at the southern limit of the Grenvillian poles and near southeastern Australia, and the second PTRM component has a pole, Hb₂, at the northern limit (Fig. 2). The inferred direction of apparent polar

wander (APW) is from south to north^{1–3}. As a reasonable approximation in this first attempt at the direct dating of multi-component TRMs from orogenic belts, we used field blocking temperatures estimated by the conventional static temperature method^{8,11,12} rather than the kinetic temperature approach developed by York^{13,14} that is formally analogous to the approach used here with isotopic closure temperatures. From the theoretical curves of Pullaiah and others¹¹, we estimate that the bulk of the Hb₁ PTRM component carried by magnetite was acquired in the range 520 – 570 °C and that the bulk of the Hb₁ component carried by hematite, if PTRM, was acquired in the range 600 – 650 °C. We thus assign a probable maximum acquisition range for the Hb₁ component of 520 – 650 °C by the static method. Similarly, field temperatures of not more than 250 °C could explain the laboratory blocking temperatures up to 450 °C for the Hb₂ PTRM carried by a titanomagnetite. We thus have an ideal situation for dating, having rocks with PTRMs acquired ~ 300 °C apart and with palaeopoles about 60° apart latitudinally straddling the entire group of Grenvillian palaeopoles.

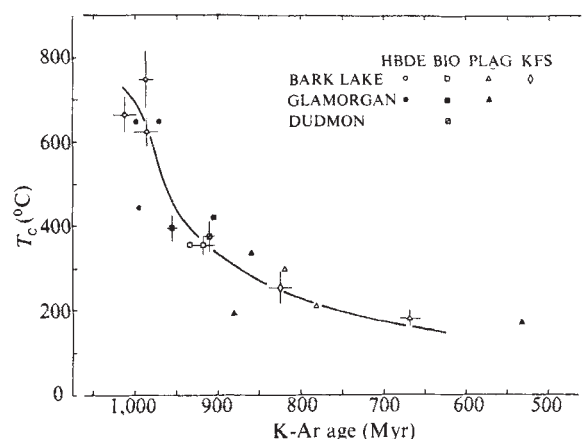


Fig. 1 An isotopically derived cooling curve for the Haliburton area using revised decay constants¹⁶. A cooling rate of 5°Myr^{-1} was assumed in Dodson's formula for all but the three rightmost points, for which $0.5^\circ \text{Myr}^{-1}$ was chosen. The error bars are 1σ .

We have dated six hornblendes, five relatively coarse biotites, six plagioclases and one potassium feldspar. Experimental details will be described elsewhere. All of the biotites and four of the hornblendes as well as the K-feldspar gave excellent plateaus over at least 90% of the released Ar. There were also significant age differences among the biotites, even from the same intrusions, and between the biotites and hornblendes. These remarkable facts can best be interpreted as due to a slow-cooling history and are least compatible with a history comprised of a sequence of episodic thermal events. Such events cannot partially reset coarse biotites and the sensitive K-feldspar without leaving anomalous and irregular age spectra¹⁵. One of the interesting discoveries in this project was that plutonic plagioclases, previously considered unreliable K–Ar clocks, yield plateau segments in the laboratory temperature range of ~ 700 – 900 °C and that these plateaus have chronological significance. Specifically, in the only sample with coexisting K-feldspar and plagioclase, the plateaus for these minerals are concordant. In calculating the diffusion coefficients for plagioclases, we have treated the Ar corresponding to the plateau segments as an independent phase. We used two conservative criteria to classify Ar closure temperatures as reliable or unreliable. First, a plateau consisting of at least five consecutive gas fractions must exist. Second, the diffusion coefficients corresponding to at least these five steps must lie on a statistically well defined straight line in an Arrhenius diagram.

The overall pattern of closure temperatures shown in Fig. 1 is internally consistent with a slow cooling interpretation and the

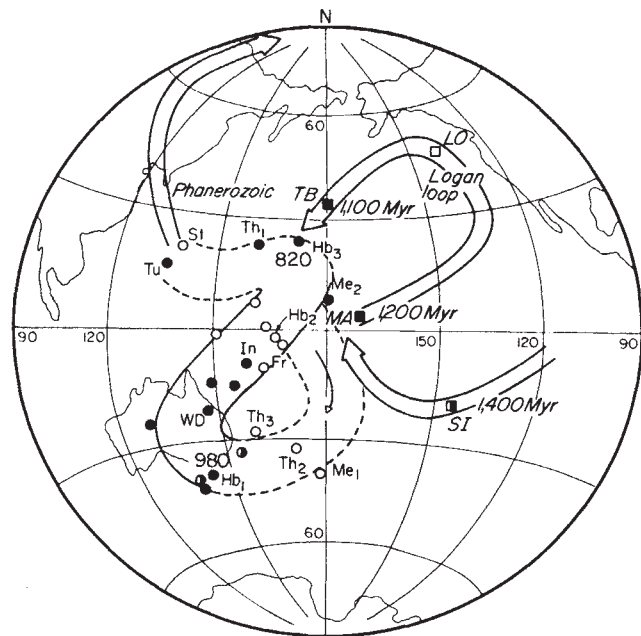


Fig. 2 A proposed Grenville Loop APW path. The calibrated part (solid) corresponds to a minimum average drift rate of 5 cm yr^{-1} . The symbols and identity of unlabelled poles are given elsewhere^{3,17}

temperatures are geologically reasonable. For example, those for the hornblendes are less than the expected maximum temperatures ($\approx 700^\circ\text{C}$) during the peak of metamorphism in this area¹⁸. Therefore, with this cooling curve, we assigned acquisition times of $980 \pm 10 \text{ Myr}$ ($520\text{--}650^\circ\text{C}$) for component Hb_1 and $\approx 820 \text{ Myr}$ ($\approx 250^\circ\text{C}$) for Hb_3 . In this way, we have calibrated the APW path for many of the Grenvillian palaeopoles.

All of the known reliable Grenvillian palaeopoles are plotted in Fig. 2 as well as poles for the Tudor gabbro (Tu) and Steel Mountain anorthosite (St) which occur within the Grenville Province. Superimposed is our proposed Grenville Loop with the calibrated track bounded by solid lines.

Although the shape, position and sense of direction of this loop have already been proposed^{19,20}, the new time limits significantly alter the previous tectonic interpretations. Our calibrated track represents a minimum average drift rate of $\approx 5 \text{ cm yr}^{-1}$, certainly more reasonable than the rates of $10\text{--}20 \text{ cm yr}^{-1}$ deduced from some previous tectonic interpretations^{19–21}. Earlier proposed Grenville Loops that lap back on themselves^{21,22} have used very approximate and indirect age assignments and should be viewed as first approximations to more refined definitions of APW paths based on direct dating procedures such as that outlined here. We must reject the two-plate hypothesis because the ages assigned to our calibrated track are much younger than required by that hypothesis ($\approx 1,000 \text{ Myr}$ north of the Equator on the northwards segment).

There is support for this calibration from other dating studies. Reynolds²³ has assigned a $\approx 910 \text{ Myr}$ date to the Frontenac Axis dikes (Fr) pole, previously thought to be $\approx 690 \text{ Myr}$. Dallmeyer²⁴ has placed the Indian Head (In) pole at $\approx 900 \text{ Myr}$. Dallmeyer and Sutter²⁵ assigned a date of $980\text{--}1,010 \text{ Myr}$ to the Whitestone diorite (WD) pole. Although the confidence ovals for the Hb_1 and WD poles overlap slightly, by interpolation in Fig. 2, the WD magnetisation has probably been acquired in the $900\text{--}980 \text{ Myr}$ interval. The main cause of the apparent discrepancy between this acquisition time and that suggested by Dallmeyer and Sutter is their choice of a much lower closure temperature ($500\text{--}550^\circ\text{C}$) for their hornblende (see ref. 26). We

think such estimates from the literature are only first approximations (they ignore sample differences, for example) and may yield only lower limits to this temperature.

The dashed, southwards track is uncalibrated but does contain poles of probable age $1,000\text{--}1,100 \text{ Myr}$ such as the Cardenas Lavas and Pikes Peak Granite poles²¹, those from the Jacobsville sedimentary formation²⁰ as well as those of the Nonsuch shale and the Freda sandstone²⁷. Pole Me_1 from the Mealy Mountain anorthosite⁴ and poles Th_3 and Th_2 from the Thanet gabbro in the Hastings Basin¹⁷, if TRM components, are predicted from Fig. 2 to have acquisition times of $\approx 980\text{--}1,020 \text{ Myr}$. The dashed segment between Hb_3 and Tu is entirely speculative because no reliable date can be assigned to Tu or St. Although the palaeomagnetic literature often quotes a 680 Myr date²⁸ for Tu, the original authors²⁸ emphasised their uncertainty concerning the event represented by this date. A further complication is the presence of North American Cambrian poles within a swath roughly from Tu to Fr^{21,29}. We are therefore unsure how an APW path should be constructed after Hb_3 .

We reject the Hadrynian Track of Morris and Roy¹⁹ and the Hadrynian Loop of Irving and McGlynn²¹ because not only are the dates of some of their critical rock units very suspect (Tudor gabbro, Franklin diabase and Aillik dikes) or incorrect (Frontenac Axis dikes), but also the time of acquisition of the Stoer Group magnetisation may be quite different from the precise 970 Myr (new decay constant) Rb–Sr date. Rb–Sr dates on sedimentary rocks are ambiguous^{30,31}.

In conclusion, through the exploitation of the $^{40}\text{Ar}/^{39}\text{Ar}$ incremental heating technique as both a diffusion experiment and a method of dating, we have calculated a cooling curve for the Haliburton intrusions and thereby have calibrated the northward track of a proposed Grenville Loop. The assigned dates are incompatible with the collisional or two-plate hypothesis as an explanation of the displaced Grenvillian palaeopoles. This is not to say that such a collision did not occur or did not influence the so-called Grenville Orogeny but rather that it has not been recorded palaeomagnetically. What is recorded is a translation and rotation of Grenvillia and Interior Laurentia²¹. The role of any such collisional process is likely to have been similar to that proposed by Dewey and Burke³² with a collisional zone south-east of the Grenville Province and now hidden under the Appalachians.

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Pore water sampling in anoxic carbonate sediments: oxidation artefacts

ANY exposure of anoxic estuarine sediments to the atmosphere during sample handling and processing can lead to decreased values of ferrous iron¹, reactive phosphate² and reactive silicate³ in the pore waters of these sediments. Thus, the mishandling of anaerobic sediments and their pore fluids will in turn lower calculated diffusional fluxes of these compounds from the sediments into the overlying water. To date, this oxidation effect has only been documented for clastic sediments rich in iron. We present data here which indicate that a similar phenomenon occurs when anoxic iron-poor, carbonate-rich sediments are exposed to an oxidising milieu.

A core was taken during June 1978 from a shallow water sub-tidal area in Coot Bay on the northeastern shore of Bermuda. Previous data^{4,5} collected in the summer of 1977 indicated that the sediments were anoxic and alkalinity and nutrient regeneration was occurring. The core was obtained by hand by carefully pushing a 6.6 cm i.d. polycarbonate core liner into the sediment.

On retrieval, the core was placed into a polyvinyl chloride core carrier, purged with nitrogen and returned to the Bermuda Biological Station for Research. The time between the taking of the sediment sample and the initiation of the pore water extraction in a nitrogen-filled glove bag was ~15 min.

The core was extruded in the glove bag under nitrogen and sectioned into six 10-cm segments. While in the glove bag, the core sections were cut in half vertically using a Teflon coated spatula. One half of the six core sections was placed into a 500 ml linear polyethylene (LPE) centrifuge bottle which had been previously cleaned for trace metal analysis⁶. The bottles were centrifuged at 10,600g at exactly *in situ* surface sediment temperatures under nitrogen.

This set of centrifuged samples was placed back in the nitrogen-purged glove bag and filtered through pre-cleaned 0.4- μ m Nuclepore filters³. The filtered samples (10 ml) were then pipetted into a 60-ml conventional polyethylene (CPE) bottle and within 30 min were analysed for pH and titration alkalinity. The titrated sample was later analysed for sulphate, calcium, phosphate and reactive silicate. The remainder of the filtered sample was decanted into a 60-ml CPE bottle which had been cleaned as described by Patterson and Settle⁶ and immediately acidified to 1% with concentrated Ultrex nitric acid for later trace metal analysis.

The other set of samples was processed in a similar manner except for two important differences: (1) the samples were removed from the N₂ atmosphere and exposed to the atmosphere of the laboratory for 10 min before being placed in the centrifuge bottles; (2) the filtration and pipetting steps were carried out in the laboratory atmosphere instead of in the glove bag. The filtering and pipetting of the supernatant pore water took ~5 min for each sample and while one sample was being processed the others were kept in the glove bag under nitrogen. Therefore the maximum atmospheric exposure for each sample was 75 min.

Phosphate analyses were run on a Technicon Auto Analyzer II system⁷ after a 2:1 dilution. The differences between duplicate dilutions never exceeded 2%. Reactive silicate was

analysed using the method of Strickland and Parsons⁸ modified for pore water by Presley⁹. Replicate analyses indicate a standard deviation of $\pm 1 \mu\text{M l}^{-1}$. Total reactive iron was analysed colorimetrically¹⁰ using the Technicon Auto Analyzer II system¹¹ with a coefficient of variation of $< \pm 0.05 \mu\text{M l}^{-1}$. The samples were never frozen and all the analyses were carried out within 5 weeks of sample collection.

We found that there was a decrease of Fe of 81% in the top 25 cm of the core when the samples were exposed to laboratory air compared with the subsamples processed under nitrogen (Fig. 1). These two sections have iron concentrations $> 1.80 \mu\text{M}$ ($100 \mu\text{g l}^{-1}$). Below this concentration the dissolved iron is apparently not affected by the atmospheric exposure. The dissolved phosphate data parallel the iron in that the greatest differences between the nitrogen processed samples and those exposed to the laboratory atmosphere are in the two uppermost core sections (Fig. 1). There is also a significant decrease in the PO_4^{3-} concentration in the lowest core section where the iron concentration is little affected. The reactive silicate values, with the exception of the 35-cm depth sample, are all lower in the laboratory exposed samples. There were no significant differences in the concentrations of pH, SO_4^{2-} , Ca^{2+} , Mn^{2+} and titration alkalinity between the nitrogen processed and the laboratory exposed subsamples.

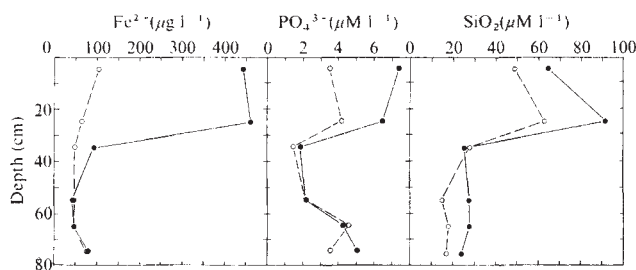


Fig. 1 Pore water concentrations of ferrous iron (Fe^{2+}), phosphate (PO_4^{3-}) and silicate (SiO_2) for a core from Coot Bay, Bermuda. ○, Samples processed after atmospheric exposure; ●, samples processed under N₂.

It seems that there is a critical dissolved iron value where laboratory exposures of as long as 75 min has no effect on the total dissolved iron concentration in the pore waters. Below this critical iron concentration, phosphate is also little affected by the presence of oxygen. The oxidation of Fe^{2+} to Fe^{3+} is very rapid at neutral pH (refs 12, 13). Whether this dissolved iron is in a physicochemical state that is unaffected by the increased partial pressure of oxygen is unknown. The iron may be associated with dissolved organic matter¹⁴ (DOM) and the kinetics of its oxidation may be much slower than that of ionic Fe (ref. 15). It is interesting that pore water iron data collected before the knowledge of oxidation effects tend to show 'residual' dissolved iron values close to $100 \mu\text{g l}^{-1}$ (refs 16, 17).

Reactive silicate, however, is continually lost by laboratory atmospheric exposure even when the dissolved iron is apparently not being oxidised. These data indicate that the mechanism of silicate removal may not be directly related to the oxidation of Fe^{2+} . It is not known what governs the loss of silicate during oxidative handling and processing, nor whether the removal of SiO_2 without the oxidation of Fe^{2+} occurs only in calcareous sediment.

The data from the top 25 cm of core indicate that for every $10 \mu\text{M}$ of iron removed during oxidation $4.5 \mu\text{M}$ of phosphate is lost. More phosphate per mol of iron is lost in these calcareous sediments than from the anoxic clastic sediments studied by Loder *et al.*³. This may be related to the exposure time of the pore water sample to the oxygen atmosphere rather than the effective scavenging potential of the iron hydroxide formed. The iron-rich clastic sediments of Loder *et al.*³ were only exposed for a period of 12 min. The Coot Bay data indicate

that an average of 28% of the reactive silicate is lost during the conditions of the experiment. This is double that found by Loder *et al.*³ for clastic sediments.

Our experiments and those of Bray *et al.*², Troup *et al.*¹ and Loder *et al.*³ emphasise the importance of proper sample handling if meaningful nutrient data from anoxic pore waters are to be obtained. Our data indicate that decreases of phosphate and reactive silicate in pore waters from anoxic carbonate-rich sediments do occur unless proper care is taken to ensure the lack of atmospheric exposure. Because pore water data are of extreme importance in understanding diagenetic pathways in marine sediments,^{18,19} and pore water exchange with overlying water may be extremely important in the fluxes of nutrients and trace metals,²⁰⁻²² the collection of pore waters from reducing sediments must be accomplished with great care, if valid geochemical models are to be produced.

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A pre-2,000 Myr old granulite facies metamorphosed evaporite from Caraiba, Brazil?

THE identification of the first occurrence on Earth of sediments of evaporitic origin formed from seawater is obviously important not only for the study of the chemical evolution of ocean water but also in understanding the development of common sedimentary rock assemblages and the stabilisation of cratons. Until recently^{1,2} confirmed pre-2,000 Myr old evaporites were unknown and indeed Ronov³ maintained that they did not exist. Pyroxene granulite facies metamorphosed evaporites are also extremely rare^{1,4} and thus a pre-2,000 Myr old example is important. We give here a brief account of the mineralogy of some of the rocks found in the Caraiba mine area of Brazil which suggests that evaporites existed earlier than previously recognised and were associated with dolomitic rocks.

The Caraiba copper deposit of Brazil is in the north of the state of Bahia, 9°52' S, 39°52' W (Fig. 1) being rich in chal-

copyrite and bornite and is associated with a sequence of high grade gneisses, pyroxene granulites, migmatites and possible meta-evaporites which together form the Caraiba supergroup, set in the eastern margin of the Sao Francisco craton. Granite gneisses which cut these high grade rocks are about $2,050 \pm 70$ Myr by Rb-Sr age determination, corresponding to the Transamazonian orogeny, so that the earlier rocks are older than this date and some charnockitic types in the Caraiba area have Rb-Sr ages of 2,900 Myr so that the metasediments could well be Archaean.

The predominant rock type in the Caraiba mine area is enderbite charnockite with biotite and this encloses the calc silicate proposed meta-evaporites but other rocks, such as granite gneisses, basic and ultrabasic granulites and pegmatoids come into contact with the possible meta-evaporites which are predominantly calc silicate diopsidites. Subordinate diopside-forsterite marbles, anhydrite-diopside $\pm$ K feldspar granulites also occur. Dolomite has not been identified in the carbonate

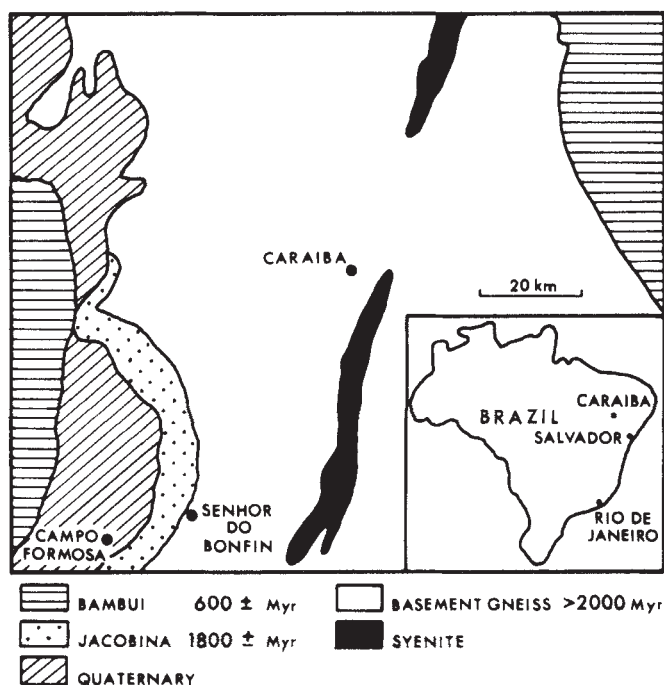


Fig. 1 Geological sketch map of the Caraiba area.

bearing rocks. Only the diopsidites outcrop, the anhydrite-bearing rocks being obtained from drill-cores. Scapolite is present in some samples. The principal host rock for the chalcopyrite-bornite mineralisation is hypersthene, and the mineralisation is thought to be magmatic, but the calc silicate rocks in contact with mineralised ultramafic or mafic rocks usually have some mineralisation. The occurrence of xenoliths of diopsidites in the hypersthene confirms the older age of the diopsidites.

Samples range from the principal assemblage which is pure diopside, sometimes with green spinel, accessory apatite and sphene through dominantly two phase diopside-anhydrite rocks with variable amounts of phlogopitic mica, olivine (forsterite), microcline, sphene and grossular garnet to samples rich in calcite. Sometimes diopsidite grades into plagioclase and sphene bearing rocks.

A coarse (3-5 mm) granular specimen with 51.6% diopside, 47.5% anhydrite and 0.9% phlogopitic mica contains nearly colourless diopside ($2V_x 55\frac{1}{2}$; $Z \wedge \gamma 41 \pm 1^\circ$) with an unzoned composition of 54.10% SiO_2 , 1.79% Al_2O_3 , 16.74% MgO , 24.18% CaO and 2.07% FeO (all Fe calculated as FeO), total 99.51% determined by electron microprobe analysis using energy dispersion. This is a composition close to pure diopside with $\text{Ca}_{0.97}(\text{Mg}_{0.91}\text{Fe}_{0.06}\text{Al}_{0.05})_{1.02}\text{Si}_{1.97}\text{Al}_{0.03}\text{O}_6$, and contains

the low Al typical of many metamorphosed calc silicate rocks. The anhydrite ($2V, 42^\circ$) has excellent cleavages and repeated lamellar twinning, and does not contain more than a trace of Sr. Such a rock, with only about 1% Al_2O_3 was clearly originally almost free of any clay or aluminosilicate minerals and was probably largely gypsum or anhydrite with diagenetic or detrital silica and perhaps a trace of illite. At one horizon olivine-anhydrite diopside samples occur with $Fe_{0.4}$.

Other samples are finer grained and contain grey, non-perthitic microcline which probe analysis shows to be nearly pure $KA1Si_3O_8$, accessory sphene containing ~1.5% Al_2O_3 , accessory pyrite and diopside with similar low Al_2O_3 (1.73%) to the above but with more TiO_2 (0.35%) and FeO (5.20%). The extreme end-member contains no anhydrite and is a fassaite-calcite-phlogopitic mica (mol $Fe/(Fe+Mg)=0.21$) assemblage that clearly contained much original aluminosilicate, presumably at least in part illite. the pyroxene in such samples varies in composition but 49.4% SiO_2 , 5.8% Al_2O_3 , 0.4% TiO_2 , 13.2% MgO , 5.1% FeO (total), 0.5% MnO , 24.4% CaO is typical, giving $Ca_{0.98}(Mg_{0.74}Fe_{0.16}Mn_{0.01}Ti_{0.01}Al_{0.11})_{1.03}Si_{1.85}Al_{0.15}O_6$. This clearly reflects both the higher Al content of this rock, which is demonstrated by the quantity of mica (10%) and its Al_2O_3 content (16%), and the high grade of metamorphism that facilitated the incorporation of substantial Al in the pyroxene. This assemblage clearly arises from an impure siliceous or silicified dolomite. In view of the predominance of diopside the commonest original sediment was presumably dolomite.

Other samples near the calcite-pyroxene rich assemblages contain a highly altered unidentified sheet-like mineral containing about 38% SiO_2 , 20% Al_2O_3 , 24% CaO with 4% $MgO+FeO$, and now partly replaced by calcite and small (0.04 mm diameter) euhedral nearly pure grossular garnet of average composition 40.02% SiO_2 , 22.14% Al_2O_3 , 37.57% CaO , 0.75% MnO and less than 0.20% FeO and MgO with a slightly MnO enriched margin (0.91%) compared with the core (0.57%). Other grains of this highly altered mineral are replaced by clinozoisite.

These preliminary results, obtained with an electron microprobe, are under further investigation, but they may support the recent indications^{1,4} that evaporites existed earlier than previously generally recognised³ and were associated with dolomitic rocks, a common association in most younger evaporite deposits. However, it is possible that the anhydrite could have formed from dolomite by SO_2 attack, the SO_2 being derived from oxidation of chalcopyrite to bornite and magnetite during the metamorphism, for some magnetite is ubiquitous. Post metamorphic oxidation is notably lacking and fresh sulphides occur in outcrops. Accordingly, the evaporitic origin is not certain.

Any original halite would obviously have migrated from such a high grade metamorphic position but if the deposit was originally a sabhka-type evaporite there may not have been very much halite. The olivine, spinel and calcite would be derived from dolomite breakdown in well-known reactions.

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Silhouette photography of oceanic zooplankton

NO adequate method has been available to characterise a sample of zooplankton quickly and accurately. As Mullen noted¹, although relatively rapid, measures of standing crop such as displacement volume, dry weight, carbon content and electronic size spectra do not distinguish clearly between animals and plant or detrital material, much less between herbivores and carnivores. Direct microscopic enumeration has remained the only approach giving information detailed enough for many ecological studies. Unfortunately, this method is time-consuming, it cannot be undertaken routinely at sea, and it is feasible only after chemical agents have been added to 'fix' and then 'preserve' the sample. With time, plankton samples always deteriorate in preservatives, although the degree of this effect varies with species. We report here that a little-known photographic technique—high-speed silhouette photography^{2,3}—can be used to analyse live zooplankton samples at sea.

The photographic system we used consisted of 8×10 inch Eastman fine-grain positive film 7302, an electronic flash with a xenon bulb (E.G. & G., Inc., FX6A) and a pulse duration of 3×10^{-6} s, and a plastic tray. Lamp-to-film distance was ~1.0 m. Immediately after collection, whole samples or aliquots of particularly dense samples were poured to a depth of about 1 cm into the plastic tray directly on top of the film. A glass plate was used to seal the top of the tray when seas were rough. One flash in a darkened room exposed the film. Samples were then preserved in the usual fashion (8% buffered formalin, pH ~8.2). Because of the shortness of the flash, action was stopped and results were unaffected by the motion of the ship or the subjects. Essentially any container and any powerful electronic flash could be used. The latter should be arranged to approximate a point-source as closely as possible. We housed the small-diameter xenon bulb in a non-reflective tube.

Fig. 1 Actual-size silhouette of a live zooplankton sample collected in the Sargasso Sea.



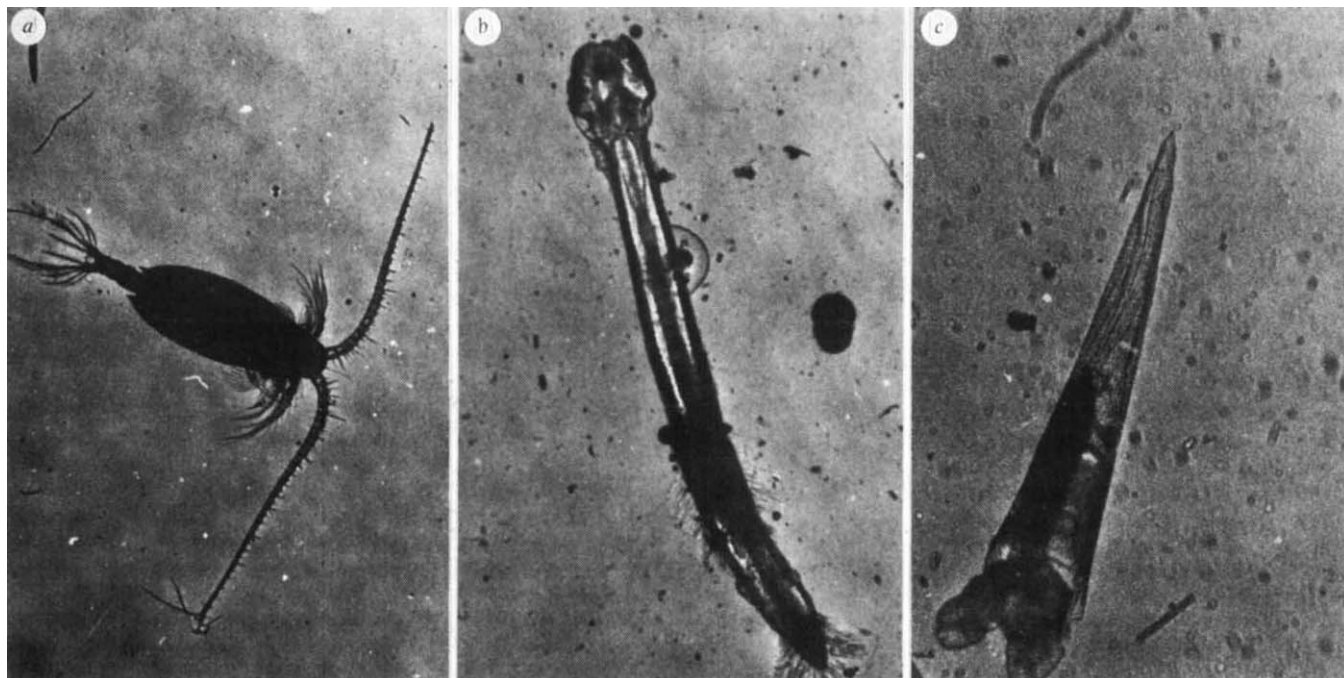


Fig. 2a Enlarged silhouette of a copepod, *Undinula vulgaris* female, ~2.5 mm. **b**, Enlarged silhouette of a chaetognath, *Pterosagitta draco* ~8 m. A fish egg lies just beyond the body trunk. **c**, Enlarged silhouette of a shelled pteropod, *Styliola subula*, ~2 mm.

Figure 1 is the contact print of a negative produced as described above. The sample was collected between 0 and 89 m depth in the southern Sargasso Sea in February 1978 and represents 443 m³ of seawater filtered through the 0.333-mm mesh of a MOCNESS net system⁴. The relatively large leaf-like animal is an eel larva at the leptocephalus stage.

Such negatives were generally examined at sea by placing them between two glass plates and counting random transects but, also, we have projected them on a viewing screen. To be enumerated in a negative, the density of organisms first would be considerably reduced by subsampling. Because the area of a negative corresponds to a known volume of ocean, it can be used to characterise quantitatively the plankton of a region if resolution were sufficient.

The resolution obtained by our optic-less imaging technique was more than adequate for this purpose. It was possible to make generic and, occasionally, specific identifications of specimens in many cases. Figure 2a-c shows enlargements of individual animals from actual-size negatives such as Fig. 1. The animals were alive when they were photographed. The copepod in Fig. 2a was moving rapidly and erratically. The chaetognath in Fig. 2b is easily identified even though the collarette is nearly gone, as frequently occurs in net collections. The pteropod mollusc in Fig. 2c has its wing-like flaps partly extended in their swimming position. The tissue of a preserved specimen typically would have contracted into an indistinguishable lump. A negative such as Fig. 1 could not have been obtained by conventional photographic or photomicrographic techniques, which do not give the breadth of the field of view and the depth of focus required to accommodate the entire sample, nor the high resolution we obtained despite ship and animal motion.

The rapidity, ease, and low cost of silhouette photography of plankton make it potentially useful to field workers with limited facilities. It can produce a permanent record of a live sample, which is unobtainable at present by any other means. Most important, the technique is non-destructive. Preservatives can be added after the image is recorded, and the sample can be sorted and examined on shore.

An *in situ* silhouette camera system designed to assess zooplankton patchiness has already been tested and a programme has been initiated to fully automate enumeration of our negatives by means of computer image-analysis techniques.

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Drinking behaviour induced by intracerebroventricular administration of enkephalins to rats

INTRACRANIAL administration of substance P to rats inhibits drinking induced by water deprivation, sodium-chloride load, and intracerebroventricular (i.c.) injection of angiotensin II or carbachol¹. It has been suggested that substance P is a neurotransmitter used in primary afferent fibres terminating in the spinal cord²⁻⁴, but recent findings suggest also that, at least in mice, substance P may release endogenous opioids and thus produce analgesia⁵. If cerebral effects of substance P are, at least in part, due to the release of endogenous opioids, these substances might also possess an antidiipsogenic effect. We

describe here experiments in which we observed that enkephalins administered into the lateral ventricles of rats inhibited drinking induced by water deprivation or by i.c. injection of angiotensin II. We thus provide evidence that enkephalins have a potent and reproducible antidipsogenic effect in the rat.

In a preliminary operation, i.c. cannulae (o.d. 0.6 mm) were implanted stereotactically in male Wistar rats. The animals weighed 250–300 g and were under Equithesin anaesthesia (3 ml per kg). They were allowed at least 10 d before testing began. Injections were made in conscious rats through a stainless-steel injector (o.d. 0.3 mm) temporarily inserted into the guide cannula. The drugs were dissolved in Tyrode solution and injected in volumes of 1 μ l. Drinking was induced by a 16-h period of water deprivation or i.c. injection of angiotensin II, 100 ng per rat. The drugs used were angiotensin II (AII, synthetic 5-isoleucine-angiotensin II; Calbiochem), (Leu⁵)-enkephalin (LEN), (Met⁵)-enkephalin (MEN) and (D-Ala², D-Leu⁵)-enkephalin (AEN) (all Wellcome).

In AII-treated rats, LEN and MEN inhibited drinking, but the effect required large amounts of peptide (100–200 μ g per rat). Drinking inhibition was apparently dose dependent (Fig. 1a, b) and, as indicated in Fig. 1d, when latency between drug injection and the beginning of water intake was recorded, a clear-cut inhibition was also seen at relatively low doses (25–50 μ g per rat).

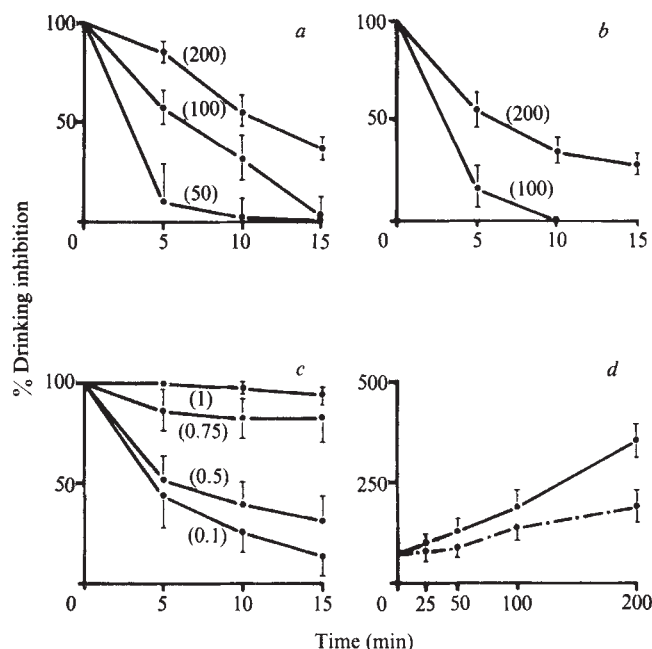


Fig. 1 Angiotensin II-treated rats. Inhibitory effect of (Met⁵)-enkephalin (a), (Leu⁵)-enkephalin (b) or (D-Ala², D-Leu⁵)-enkephalin (c) on water intake induced by i.c. injection of angiotensin II, 100 ng per rat. In parentheses, doses in μ g. Latency time after i.c. administration of (Leu⁵)-enkephalin (continuous lines) or (Met⁵)-enkephalin (dotted lines) is shown in d. Points and vertical bars represent means $\pm$ s.e. of 6–8 experiments.

AEN was about 2,000–4,000 times as active as the naturally occurring enkephalins LEN and MEN. In fact, a complete inhibition was attained with 1 μ g of AEN (Fig. 1c), whereas 0.5 μ g of the peptide had almost the same inhibitory effect as 100 μ g LEN and 200 μ g MEN.

In water-deprived rats, LEN and MEN inhibited water intake (Fig. 2a). The effect was short lasting and, again, required large amounts of peptide (200 μ g per rat). As before, AEN was more effective than LEN and MEN: 10 μ g completely inhibited drinking for more than 60 min, and a clear-cut inhibitory effect, lasting more than 30 min, was elicited by doses as low as 100 ng (Fig. 2b). Lower doses (10 ng) were still effective, but difference from controls was not statistically significant.

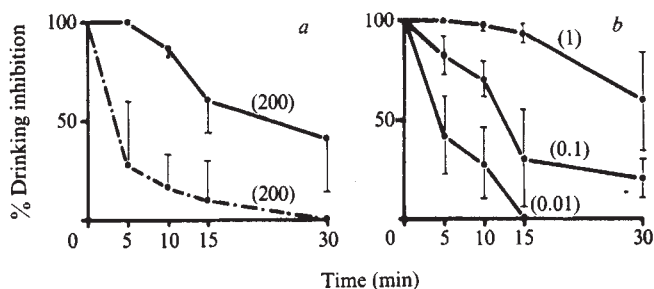


Fig. 2 Water-deprived rats. Inhibitory effects of (Leu⁵)-enkephalin (a, continuous lines), (Met⁵)-enkephalin (a, dotted lines) or (D-Ala², D-Leu⁵)-enkephalin (b) on water intake induced by a 16-h period of water deprivation. In parentheses, doses in μ g. Points and vertical bars are means $\pm$ s.e. of 6–8 experiments.

In both kinds of experiments, animals which received LEN or MEN, 200 μ g per rat, were immobile and did not react promptly to tactile and acoustic stimuli. This 'depressive' effect lasted about 5 min and disappeared before the antidipsogenic effect. Rats which received these substances at relatively low doses (25–50 μ g) as well as AEN at doses of 1 μ g or less did not react in this way. At these dose levels, enkephalins did not produce any nonspecific behavioural modification or modify food intake in rats trained to eat their daily allowance of food during a 2-h period, between 2100 and 2300 (Fig. 3). Naloxone, 2,000 μ g per kg, subcutaneously, reduced but did not completely abolish the antidipsogenic effect of the peptides.

The present data provide evidence that i.c. administration of enkephalins elicits in the rat an antidipsogenic effect which is potent, reproducible and dose dependent. This effect might be considered specific and not simply due to the depressive activity of enkephalins. In fact, it was evoked even by doses which did not affect the rats' nonspecific behaviour, as in the case of AEN, which was effective at doses as low as 10 ng, and was still evident after the 'depressive' response had disappeared, as in the case of LEN, 200 μ g, which, in water-deprived rats, significantly inhibited drinking for more than 30 min. Moreover, water intake, but not food intake, was modified by the peptides.

The different potency of the naturally occurring enkephalins, on the one hand, and AEN, on the other, may be accounted for by the different sensitivity of the latter to enzymatic hydrolysis, as a consequence of replacing Gly₂ by D-alanine⁶.

The results of these experiments, although confirming our working hypothesis, give us no conclusive indication of whether substance P inhibits drinking by means of enkephalin release, nor any indication of the possible physiological significance of the antidipsogenic effect of enkephalins.

Thirst is an unpleasant sensation which may be so severe as to be regarded as a suffering and, in a wide sense, as pain. Thus, it is

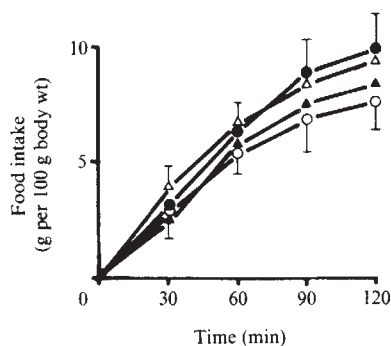


Fig. 3 Food intake in rats trained to eat their daily allowance of food during a 2-h period, between 2100 and 2300 (ref. 1). Controls ($\blacktriangle$) received 1 μ l of Tyrode solution, i.c.; treated rats received 1 μ l of Tyrode solution containing fully antidipsogenic doses of LEN ($\bullet$, 200 μ g), MEN ($\circ$, 200 μ g) or AEN ($\triangle$, 1 μ g). Points and vertical bars are means $\pm$ s.e. of 5–7 experiments.

not entirely impossible that the analgesic effect of enkephalins may be involved in the mechanism(s) of water intake control. However, the fact that the antidiipsogenic effect of enkephalins is reduced but not abolished by naloxone suggests that it is only in part, if at all, due to activation of opiate receptors.

These are entirely speculative hypotheses. Experiments are being carried out in our laboratory to check their validity.

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Hyperpolarisation and depression of slow synaptic inhibition by enkephalin in frog sympathetic ganglion

EVIDENCE is accumulating to support the concept that the pentapeptides Met-enkephalin and Leu-enkephalin, the endogenous ligands for the opiate receptor, function as neuromodulators or neurotransmitters^{1,2}. In the central nervous system the effects of enkephalins are complex and difficult to analyse, and their mode of action at the neuronal membrane level is still poorly understood. The most prominent action of enkephalins in the mammalian brain is depression of neuronal firing rate and it has been suggested that these peptides are inhibitory transmitters^{1,2}. An increase in firing rate by enkephalins has, however, also been reported³. The response of central neurones to several putative transmitter substances is depressed or enhanced by enkephalins, suggesting a postsynaptic action^{4,5}. It has also been shown that enkephalins suppress the K⁺-induced release of noradrenaline⁶, dopamine⁷ and acetylcholine⁷ from rat brain slices, indicating a presynaptic effect. The firing of myenteric neurones in the guinea-pig ileum is inhibited by enkephalins. This inhibition is probably due to a direct postsynaptic action of the enkephalins resulting in a hyperpolarisation of the neuronal membrane⁸. Most of the effects of enkephalins are antagonised by the specific opiate antagonist naloxone. Morphine produces effects very similar to those of enkephalins. Because of the possible role of enkephalins in inhibitory synaptic transmission, we studied the effects of Met-enkephalin on slow synaptic inhibition in frog sympathetic ganglion. We report here that Met-enkephalin causes a depression of the slow inhibitory postsynaptic potential (i.p.s.p.), probably mainly by a presynaptic action, and induces a hyperpolarisation of the ganglionic neurones. Both effects are antagonised by naloxone.

The experiments were carried out on the 9th or 10th paravertebral sympathetic ganglion of the frog (*Rana temporaria* and *R. esculenta*), using the sucrose gap technique⁹. Slow i.p.s.ps were elicited by repetitive stimulation of the ramus to the 8th ganglion. The simultaneously evoked fast excitatory postsynaptic potentials were selectively blocked by a low concentration of nicotine (30 μ M) which was continuously present in the perfusion solution. Drugs were applied by switching from the original perfusion solution to one which differed only in its

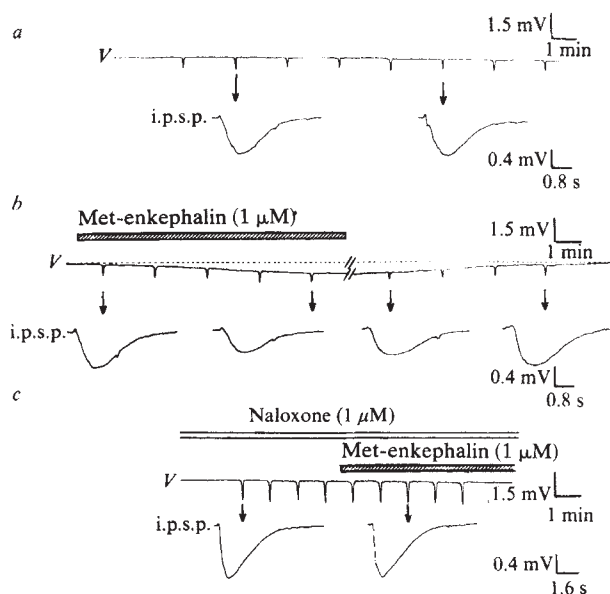


Fig. 1 Effects of Met-enkephalin on membrane potential and slow inhibitory postsynaptic potential (i.p.s.p.) in frog sympathetic ganglion. Experiments were carried out on the 9th or 10th paravertebral sympathetic ganglion using the sucrose gap technique⁹. The ganglion was continuously perfused with Ringer solution, with or without drug, containing in mM: NaCl 160; KCl 2.4; CaCl₂ 1.7 and HEPES 3.0; at pH 7.3 and temperature 21–23 °C. The postganglionic nerve trunk was perfused with isotonic KCl. Slow i.p.s.ps were elicited by repetitive stimulation of the ramus to the 8th ganglion through a suction electrode with a train of five pulses (0.1 ms; 10 Hz). Fast excitatory postsynaptic potentials were selectively blocked by the continuous presence of 30 μ M nicotine in the perfusion solution. Upper trace in a, b and c represents continuous recording of voltage across sucrose gap (V) on the strip chart recorder. Downward deflections are slow i.p.s.ps. Lower traces, samples of enlarged slow i.p.s.ps recorded with a faster time base at different times as indicated by arrows. a, Control; b, hyperpolarisation and depression of slow i.p.s.p. by application of 1 μ M Met-enkephalin as indicated by hatched bar. Dashed line in upper trace indicates baseline voltage. Both effects were reversed rapidly when the application of Met-enkephalin was stopped. c, Application of 1 μ M naloxone (open bar) completely prevented the effects of subsequent application of 1 μ M Met-enkephalin (hatched bar). Naloxone itself was without noticeable effect.

content of the drug to be studied. To detect changes in the ganglionic neurone resting membrane potential, which is reflected by the voltage across the sucrose gap, much care was taken to ensure a stable recording. In most experiments d.c. stability was better than 1 mV h⁻¹.

Application of 1 μ M Met-enkephalin consistently caused a hyperpolarisation of the ganglionic neurones. In all experiments with Met-enkephalin a small drop in voltage across the sucrose gap (0.2–0.5 mV) was observed (Fig. 1b). Simultaneously with the hyperpolarisation, Met-enkephalin produced a marked depression of slow synaptic inhibition (Fig. 1b). In six experiments the amplitude of the slow i.p.s.p. was depressed by 39.3 $\pm$ 6.5% (mean $\pm$ s.d.). Duration and time course of the slow i.p.s.p. were not significantly affected by Met-enkephalin. Both the hyperpolarisation and the depression of the slow i.p.s.p. attained a constant level 5–10 min after the enkephalin had reached the ganglion, and were completely reversed on washing with enkephalin-free solution. Reapplication of Met-enkephalin produced the same effects.

D-Ala-Met-enkephalinamide at 1 μ M caused similar effects (three experiments), but no attempt was made to compare the potencies of the two peptides. Morphine at 5 μ M also caused a hyperpolarisation together with a depression of the slow i.p.s.p. (three separate experiments). Application of 1 μ M naloxone fully reversed or prevented both the hyperpolarisation and the depression of the slow i.p.s.p. induced by Met-enkephalin (Fig.

1c). Naloxone itself had no noticeable effect. Thus, the enkephalin-induced effects can be considered to result from a specific interaction with opiate receptors. In myenteric neurones normorphine also causes a naloxone-reversible membrane hyperpolarisation which is accompanied by a fall in membrane resistance, as demonstrated by intracellular recording¹⁰. The hyperpolarisation of the sympathetic neurones reported here may be due to a similar mechanism.

There is evidence that the driving force for the slow i.p.s.p. is the difference between the membrane potential, V_m , and the K^+ equilibrium potential, E_K (ref. 11). Therefore, the depression of the slow i.p.s.p. by Met-enkephalin could be a consequence of the enkephalin-induced hyperpolarisation of the postsynaptic membrane. To test this possibility the relationship between slow i.p.s.p. amplitude and membrane potential was studied by varying the external K^+ concentration. The slow i.p.s.p. amplitude seemed to be linearly related to $V_m - E_K$ calculated with the aid of the Goldman equation, assuming standard values for the internal K^+ concentration (144 mM) and for the permeability ratio (P_{Na}/P_K , 0.04) (ref. 12). The estimated hyperpolarisation necessary to reduce the slow i.p.s.p. by 40% was much larger than the hyperpolarisation induced by Met-enkephalin, even if it is assumed that the enkephalin affects only C cells which generate the slow i.p.s.p. Thus, an additional effect seems to be involved.

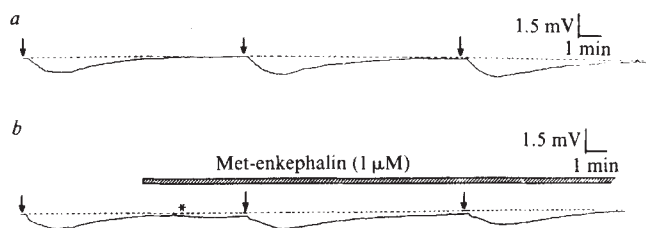


Fig. 2 Effects of Met-enkephalin on transient hyperpolarisations produced by single applications of dopamine (1 µl; 100 mM) just over the ganglion (arrows). *a*, Control; *b*, after application of 1 µM Met-enkephalin as indicated by hatched bar. Dashed line indicates baseline voltage across sucrose gap. Asterisk denotes beginning of hyperpolarisation induced by Met-enkephalin.

The slow i.p.s.p. in sympathetic ganglia is considered to be brought about by a catecholamine, probably dopamine, released from an interneurone which in turn is activated through a muscarinic cholinergic synapse¹³. To determine whether Met-enkephalin influenced the sensitivity of the ganglion to dopamine, small amounts of this substance were applied just over the ganglion with the aid of a motor-driven microinjection device. A single ejection of 1 µl of a 100 mM solution of dopamine produced a transient hyperpolarisation with a maximum amplitude of 0.5–3.0 mV in different experiments (Fig. 2*a*). Repeating the ejections at an interval of 10 min produced a constant response over a period of hours. Application of Met-enkephalin (1 µM) had only a slight effect on the dopamine response (Fig. 2*b*); in five experiments the amplitude was reduced by an average of $13.8 \pm 6.1\%$. This reduction may be caused by the enkephalin-induced hyperpolarisation of the postsynaptic membrane as discussed above. These results suggest that the postsynaptic sensitivity of the ganglionic cells towards dopamine is hardly affected by Met-enkephalin.

The most likely explanation of the depression of the slow i.p.s.p. by Met-enkephalin reported here is an inhibition of transmitter release from presynaptic nerve terminals. Such a mechanism has been postulated to explain the inhibition of the K^+ -induced release of various putative transmitter substances, including dopamine, from rat brain slices by enkephalin^{6,7}; a presynaptic localisation of opiate receptors on dopaminergic nerve terminals has been demonstrated in rat striatum¹⁴.

In conclusion, the present results show that Met-enkephalin exerts a dual effect in frog sympathetic ganglion; it induces a hyperpolarisation of the ganglionic neurones and in addition

depresses slow synaptic inhibition. The hyperpolarisation probably involves a postsynaptic site of action, whereas the depression of slow synaptic inhibition may be presynaptic in origin, due to the inhibition of transmitter release from the nerve terminals.

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mRNA species directing synthesis of milk proteins in normal and tumour tissue from human mammary gland

ONLY about one third of human metastatic breast carcinomas regress in response to hormone therapy^{1,2}, so there is a need to predict which tumours are of the hormone-dependent type. The usual method of prediction is based on the determination of hormone receptors in the tumour tissue^{2–5}. However, only about one half of the tumours containing measurable oestrogen-receptor levels respond to hormone treatment² suggesting that receptors which bind the hormone *in vitro* may not always respond *in vivo*. An alternative approach has been to look for milk proteins within the tumour or serum, as lactation is a known hormone-dependent process. Although the rationale behind this approach has been substantiated in experimental systems^{6,7} results with human mammary carcinomas are inconclusive^{8–16}, probably because of insufficient specificity¹⁷ and sensitivity of the radioimmunological protein assay on which this method depends. In addition to technical problems the immunological approach has the drawback that it can only monitor the final product of hormone action, the mature protein, and not the initial RNA transcript(s). A better approach would seem to be an assay designed to detect differences in transcriptional rather than translational activity. As a preliminary step towards this objective, we describe here the isolation of poly(A)-containing RNA from a normal lactating human mammary gland, and demonstrate the presence of mRNA species which direct the synthesis in cell-free protein-synthesising systems of human casein and α -lactalbumin. Moreover, using the same techniques we have identified the presence of mRNA directing the synthesis of α -lactalbumin in poly(A)-containing RNA isolated from two out of five mammary tumours examined.

The tissue we describe as 'normal' was obtained from a nursing mother 15 days postpartum and consisted of a large (2 g) retention cyst, histologically virtually normal, which showed

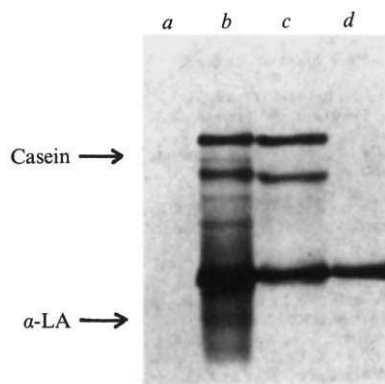


Fig. 1 Identification of casein and α -lactalbumin (α -LA) mRNAs in poly(A)-containing RNA isolated from lactating human mammary gland. A 2-g sample of frozen human lactating mammary gland (-70°C) was pulverised with a hammer, then ground to a coarse powder in a mortar and pestle with periodic addition of liquid nitrogen. The powdered tissue was then rapidly transferred to a Teflon-in-glass Potter-Elvehjem homogeniser (0.4 mm clearance) containing 20 ml of 2% (w/v) SDS, 0.5 mg ml $^{-1}$ proteinase K (Boehringer), 1 mM EDTA, 20 mM Tris-HCl, pH 7.6 and homogenised briefly at room temperature. It was then incubated at 45°C for 1 h. After adding NaCl to 0.1 M, the lysate was extracted once with an equal volume of phenol-chloroform (1:1) saturated with 0.1 M NaCl, 1 mM EDTA, 20 mM Tris-HCl, pH 7.6. After separating the phases by centrifugation (3,000g, 10 min, 15°C) nucleic acids were precipitated from the aqueous phase with 2.5 volumes of absolute ethanol at -20°C for 1 h. The precipitate was collected by centrifugation (12,000g, 10 min, 0°C) and redissolved in 11 ml of 1 mM EDTA, 20 mM Tris-HCl, pH 7.6. Solid caesium chloride (4.4 g) was added (0.4 g ml $^{-1}$) and the solution was distributed between two 12-ml capacity Beckman Ti50 centrifuge tubes, each containing a 3-ml cushion of 5.7 M caesium chloride in buffer. After overlaying with paraffin oil the tubes were centrifuged at 40,000 r.p.m. for 18 h at 20°C in a Beckman Ti50 rotor. The supernatant was removed, the bottoms of the tubes sliced off, and the RNA pellets redissolved in buffer. After ethanol precipitation, the total RNA was subjected to two cycles of affinity chromatography on oligo(dT)-cellulose¹⁸ to obtain the poly(A)-containing fraction. mRNA activity was determined in a wheatgerm cell-free protein-synthesising system¹⁸, in the presence of ^{35}S -methionine. The products were electrophoresed on Maizel polyacrylamide gels³¹ containing SDS³², and visualised by fluorography³³. Antisera to human α -lactalbumin and the major casein isolated from milk^{9,20,34} were raised in rabbits¹⁸. Immunoprecipitates were prepared and processed as described previously¹⁸. *a*, Wheatgerm products synthesised in the absence of added RNA. *b*, Proteins synthesised on the addition of poly(A)-containing RNA from lactating human mammary gland. *c*, Proteins specifically precipitated by combined antisera to human α -lactalbumin and the major casein isolated from milk. *d*, Proteins specifically precipitated by anti- α -lactalbumin antiserum alone. Arrows indicate the positions of authentic mature milk proteins isolated from milk.

lactating breast tissue with areas of severe inflammation and abscess formation, but no evidence of malignancy. This and other tumour tissue samples were deep-frozen in liquid nitrogen within minutes of excision, and then stored at -70°C before processing as described in the legend to Fig. 1.

The addition of the total cellular poly(A)-containing RNA isolated from normal human lactating mammary gland tissue to the wheatgerm cell-free protein-synthesising system resulted in a fourfold stimulation in the incorporation of ^{35}S -methionine into protein, when compared with that of the endogenous level in the absence of added RNA. Visualisation of the synthesised products by fluorography after separation by SDS-gel electrophoresis revealed the presence of three prominent bands of estimated molecular weights 26,500, 23,000 and 16,500 (Fig. 1). However, none of these products co-electrophoresed with the authentic human milk proteins (casein 25,000; α -lactalbumin 14,500), a situation we have previously reported with respect to the mRNA-directed synthesis of guinea pig milk proteins in the wheatgerm cell-free protein-synthesising system¹⁸. Further product analysis, using a mixture of antibodies raised against highly purified preparations of the major human casein component, and of human α -lactalbumin, resulted in the preferential precipitation of all three bands, whereas treatment with antisera raised against α -lactalbumin alone resulted in the precipitation of the lowest molecular weight component only (Fig. 1).

These results are consistent with the synthesis in the wheatgerm cell-free protein-synthesising system (or the reticulocyte lysate system—L. H. and R. C., unpublished observations) of a precursor form of α -lactalbumin (pre- α -lactalbumin). The situation concerning the remaining two higher molecular weight protein products is less clear. Human milk contains two caseins, the major component a well characterised protein (molecular weight 25,000), and a minor component, the κ -casein. We have purified and raised antisera against the former, although it is known that this cross-reacts immunologically with the κ -casein component¹⁹. Thus it seems reasonable to conclude that both bands represent human caseins, and that the higher molecular weight predominant protein probably represents a precursor to the major human casein, whilst the minor lower molecular weight protein probably represents the equivalent precursor to the κ -casein. However, as it is known that the major human casein is also highly phosphorylated²⁰, one cannot exclude the possibility that the anomalous mobility of the human caseins might be due to the absence of this post-translational event in the wheatgerm cell-free system. The relevance of pre-proteins and the absence of post-translation modification events in the wheatgerm cell-free system, concern the intracellular mechanisms involved in the synthesis of secretory proteins. These have been discussed in detail elsewhere with regards to guinea pig milk protein synthesis^{18,21} and will not be discussed further in the context of this paper, except to note that in common with the guinea pig¹⁸, rat²², ovine and bovine milk proteins^{23,24}, the primary human milk protein translational products are probably synthesised as pre-proteins.

Processing of human mammary tumour tissue in an identical manner produced considerably lower yields of total RNA per g of starting material (15 A_{258} units per g) than the normal lactating tissue (75 A_{258} per g), although in all tissue samples processed in this manner the poly(A)-containing RNA recovered consistently represented about 1% of the total RNA population. Addition of the tumour-derived poly(A)-containing RNA to the wheatgerm cell-free system resulted in a slight stimulation in protein synthesising activity in two out of five tumours examined (all from postmenopausal patients). Analysis of the *in vitro* synthesised ^{35}S -methionine-labelled protein

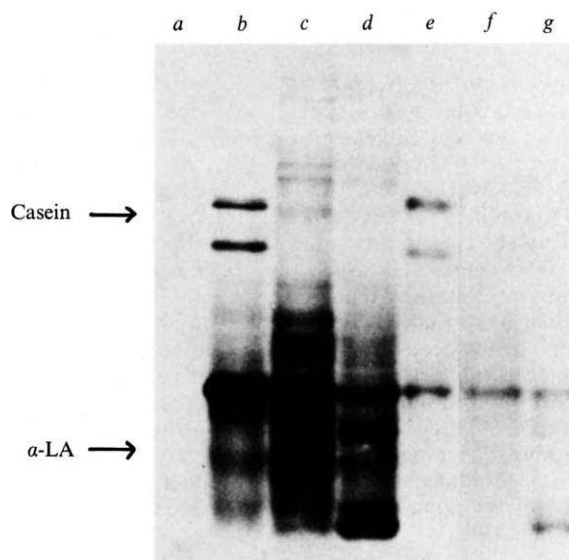


Fig. 2 Identification of α -lactalbumin mRNA in poly(A)-containing RNA isolated from human breast tumours. Experimental details as for Fig. 1, poly(A)-containing RNA being isolated from frozen breast tumours in the way described for lactating tissue. *a*, Wheatgerm products, synthesised in the absence of added RNA. *b*–*d*, Proteins synthesised on the addition of poly(A)-containing RNA from lactating human mammary gland (*b*), breast tumour 1 (*c*) and breast tumour 2 (*d*). *e*–*g*, Proteins specifically precipitated by combined antisera to human α -lactalbumin and the major casein isolated from milk; lactating human mammary gland (*e*), breast tumour 1 (*f*) and breast tumour 2 (*g*). Arrows indicate the positions of authentic mature milk proteins isolated from milk.

products (Fig. 2) showed in each case a prominent protein band which co-electrophoresed with human pre- α -lactalbumin, but no comparable bands which co-electrophoresed with the equivalent putative human pre-caseins. Antibody precipitation of the products with a mixture of antisera against both human α -lactalbumin and the major casein component confirmed that neither tumour contained significant levels of active mRNA which directed the synthesis of human caseins, though both contained mRNA which directed the *in vitro* synthesis of pre- α -lactalbumin. In addition to pre- α -lactalbumin, poly(A)-containing RNA from tumour 2 also directed the synthesis of a prominent lower molecular weight protein product (11,500), also precipitable by milk-protein antibodies. Subsequent work has shown that this is precipitated by antisera raised against α -lactalbumin, and not by antisera raised against human casein preparations.

Of the remaining three mammary tumours processed, although poly(A)-containing RNA was recovered in the expected yield, all showed inhibitory activity in a variety of cell-free protein-synthesising systems, possibly a function of low molecular weight nuclear RNA species known to inhibit cell-free protein-synthesising systems²⁵⁻²⁸.

As well as demonstrating mRNA-directed synthesis *in vitro* of human milk proteins, our results provide firm evidence for the presence, in significant levels, of mRNA directing the synthesis of α -lactalbumin but not casein, in two out of five mammary tumours examined. Both were from postmenopausal patients, one of which (T1—an adenocarcinoma) had had no children, whilst the other (T2—an invasive duct carcinoma) had had one breast-fed child. Neither had undergone either hormone therapy or chemotherapy before surgery.

α -Lactalbumin has previously been identified in the cytosol fraction of mammary tumours, using radioimmunoassay. One study in particular¹⁶ provided good evidence for the presence of α -lactalbumin in 38% of all mammary carcinomas examined. Of these, 53% of the ER-positive carcinomas contained α -lactalbumin, but only 5% of the ER-negative carcinomas, results which strongly suggest that the presence of α -lactalbumin may indicate an intact oestrogen-receptor mechanism, and may therefore provide a useful marker for identifying tumours responsive to hormone therapy. Our preliminary results support such a concept. Our approach is at present time-consuming and therefore not suitable for routine diagnostic application. However, it should prove possible to apply modern recombinant genetic techniques to this system, as has been done with both adult and fetal globin genes²⁹, thus providing in the form of a recombinant DNA molecule the basis of a highly sensitive nucleic acid hybridisation assay capable of detecting low levels of milk protein mRNA sequences in relatively small samples of breast tissue. This approach has the additional advantage that other RNA molecules which may be inhibitory in cell-free protein synthesising systems will have no deleterious effect on molecular hybridisation. The potential of molecular hybridisation has already been demonstrated by Rosen and Socher³⁰ who examined the effect of hormones on the levels of rat casein mRNA by hybridisation to a complementary DNA copy of the casein mRNA, thus by-passing any inconsistencies in radioimmunoassay technology, and the dependence of such assays on the presence of the final translational product, and not the initial product of hormone-dependent gene expression, the primary RNA transcript.

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Translocation 8;14 in an ataxia telangiectasia-derived cell line

THE incidence of lymphomas is 10,000 times greater than normal in patients with immunodeficiency syndromes¹ and also in ataxia telangiectasia (AT), which very often involves IgA deficiency². In addition to its other characteristic features, AT is usually associated with a defect in DNA repair³ and a translocation involving chromosome 14 in the peripheral leukocytes⁴. Another feature is a high prevalence of Epstein-Barr virus early antigen (EBV-EA) antibodies⁵ in the sera of AT patients. This is significantly higher than in normal human sera (<10%) and resembles that found in cases of Burkitt's lymphoma (BL). It is thus interesting that translocations involving chromosome 14 have been described in BL cells as well as in other lymphomatous cells⁶⁻⁸. This chromosome marker is also found in BL cell lines containing EBV⁹. It is not induced by EBV alone, however, because it is not found in EBV-positive, but non-lymphomatous cell lines, established spontaneously or by transformation of normal peripheral blood leukocytes¹⁰. We have therefore investigated whether in BL and AT some target cells of EBV infection might be genetically 'defective' lymphoid cells that could more easily be transformed into malignant cells (with characteristic chromosome markers) when exposed to EBV or other carcinogens. To do this we infected lymphoid cells from AT patients *in vitro* with EBV, and we report here a translocation t(8q⁻; 14q⁺) established in one of the resulting cell lines.

The translocation seems to be identical to that found in BL^{6,7}. This suggests that *in vitro* transformation of peripheral lymphocytes from a patient with a genetic predisposition to

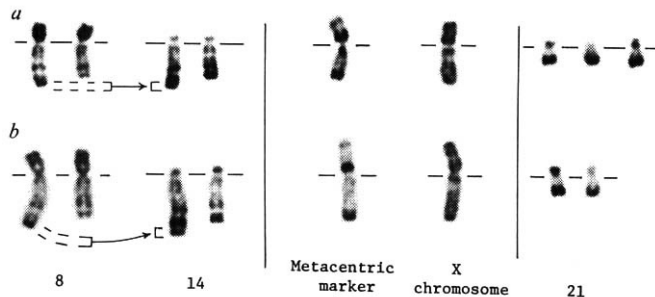


Fig. 1 R-banded chromosome rearrangements in *a*, ataxia telangiectasia- *b*, Burkitt lymphoma-derived cell lines.

chromosome damage favours the development of a chromosome rearrangement already described in lymphoid malignancies^{8,10}.

Phytohaemagglutinin-stimulated peripheral blood lymphocytes from a 17-yr-old male patient with AT were analysed by R-banding. In addition, a cell line (CB-1) was established from his blood lymphocytes by transformation *in vitro* with EBV obtained from the saliva of a patient with infectious mononucleosis according to a method previously described¹¹. Karyotypes were prepared from this cell line 6, 8 and 12 months after it had been established. Structural rearrangements were compared with those seen in a BL-derived cell line (HR1K-m) which was examined on two separate occasions, 1 month apart, and with those of a control lymphoblastoid cell line established from normal cord blood lymphocytes transformed by EBV (Cb 8-11). Chromosomes of the three cell lines were analysed using a modification of the original technique of Dutrillaux and Lejeune¹². R-bands obtained with this technique reveal abnormalities involving relatively small portions of chromosomes.

No significant chromosomal abnormalities were observed in 108 metaphases from the peripheral blood leukocytes of the patient. However, translocations involving 14q have been described in the blood of some other AT patients⁴. The major part of 14q (break point in band 14q12) is translocated to the end of the homologue 14 or chromosomes 6, 7 or X (ref. 4). As Fig. 1 shows, an AT-derived cell line shows three different chromosomal abnormalities: a translocation t(8q;14q⁺), a metacentric marker of the size of an X chromosome with a large dark band just above the centromere, and a trisomy 21. The first two abnormalities closely resemble those seen in the BL-derived cell lines, Burkitt tumour cells and other lymphomatous cells. In the control cell line (Cb 8-11) derived from normal cord blood lymphocytes transformed by EBV, chromosomes 8, 14 and 21 were normal and no metacentric marker was seen. All 35 metaphases from the AT-derived cell line contain the translocation t8;14 and the metacentric marker (Table 1). Trisomy 21 is absent only from three metaphases with 47 chromosomes. This could be interpreted as either the presence of a second clone or the loss of a G chromosome. It is interesting that although the patient had no features of the trisomy 21 syndrome, two first-degree cousins of his father had given birth to children with Down's syndrome.

Table 1 Chromosome markers in ataxia telangiectasia- and Burkitt lymphoma-derived cell lines

Cell line origin	Total no.	Photographed metaphases		
		No. containing a		
		t(8;14)	Metacentric marker	Trisomy 21
AT + EBV	35	35	35	32
BL	36	36	36	0

AT, Ataxia-telangiectasia; BL, Burkitt lymphoma (cell line HRI-Km); EBV, Epstein-Barr virus.

EBV can transform peripheral blood lymphocytes from normal donors into permanent cell lines which remain diploid for a long time after establishment. Non-random chromosome abnormalities are acquired on prolonged culture. After more than a year, gains particularly involving five autosomes (numbers 3, 7, 8, 9 and 12) and sex chromosomes have been reported in these lymphoblastoid cell lines¹³. In our investigation, chromosome rearrangements similar to those seen in BL-derived cell lines were already present at the time of the first cytogenetic analysis, 6 months after EBV transformation.

Because both cell lines come from male donors, the possibility of contamination of one cell line by another cannot be excluded by using the Y chromosome as a distinctive marker. However, they have different histocompatibility antigens (CB-1: A3AW32, B5BW35, CW2; HR1K-m: A28AW34, B15BW49, CW2). Although an unusually high incidence of chromosomal damage¹⁴ and defective DNA repair³ has been reported in AT skin fibroblasts, rates of SV40 transformation of these cells are no higher than those for normal cells¹⁵. Furthermore, marker chromosomes similar to that described here have not been identified in these fibroblasts. In our experiment, AT lymphocytes were found, after transformation *in vitro*, to have a 14q translocation which seems to be a marker of lymphoid malignancy^{4,7,8,16}.

It seems that the addition of EBV to apparently normal lymphocytes of an AT patient has revealed either a quiescent clone or an increased chromosome fragility. This is interesting because EBV transformation of lymphoid cells from normal individuals has not so far induced the characteristic chromosome 14 marker seen in lymphomatous cells¹⁰. Further studies will be necessary to characterise CB-1 as a 'lymphomatous cell line' or as a 'lymphoblastoid cell line' as proposed by Nilsson¹⁷. The role of genetic factors such as defective DNA repair, and of susceptibility to oncogenic agents in cell transformation and malignancy may thus be clarified. Cell lines derived from lymphocytes of AT patients and others with increased susceptibility to lymphoid malignancy, might become useful tools for the study of malignant transformation. Lymphocytes from other AT patients are now being transformed by EBV, and cytogenetic studies will be made to discover if a correlation exists between early chromosome rearrangements and future clinical course, with special attention to the development of lymphomas.

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Re-evaluation of the number of specific β -adrenergic receptors on muscle cells

It has been reported by Atlas, Hanski and Levitzki that muscle cells have an unusually high number of β adrenoreceptors, 80,000 per cell¹. However, we consider the pharmacological characterisation of these receptors as specific β adrenoreceptors unsatisfactory. We report here that the number of specific β adrenoreceptors in a similar but not identical muscle cell line of the same origin is 8,000 rather than their stated figure of 80,000, and is therefore comparable to that found on C6 glioma cells.

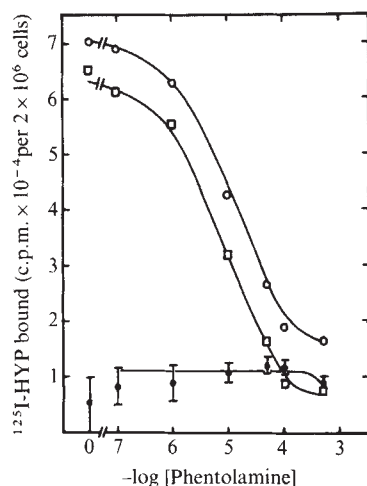


Fig. 1 The effects of phentolamine on ^{125}I -HYP binding to intact L8 muscle cells. An L8 cell line was established by serial passage of rat myoblasts²; it has properties similar to those of the L6P cell line. The cells were grown on Falcon or Nunc tissue culture plates in Dulbecco's minimal essential medium (Gibco) supplemented with 10% fetal calf serum (Gibco), under an atmosphere of 95% air, 5% CO_2 . Trypsinised, exponentially growing cells were distributed on to 6-cm plastic plates. They were then allowed to grow and recover for 48 h in fresh medium before the binding assay. In this condition the cells multiplied about ninefold. This means that if trypsin has any effect on the β -receptor density, it would affect only 10% of the cells. HYP was iodinated by the method of Maguire *et al.*⁴. 2×10^6 cells per 6-cm plate were incubated with ^{125}I -HYP (125,000 c.p.m. or 25 pM) in 2 ml of phosphate-buffered saline (PBS) and various concentrations of phentolamine in the absence (○) or presence (□) of 10^{-7} M (–)propranolol. Prewashing of the plates, their incubation with the radioactive ligand and further washing were carried out as previously described². The washing covers a total period of 60 min at 37 °C. Such a prolonged washing has negligible influence on the dissociation of ^{125}I -HYP specifically bound but markedly decreases the nonspecific binding (not shown). This observation is compatible with what is found in the literature concerning ^{125}I -HYP binding. The dissociation of ^{125}I -HYP specifically bound is observed only when an excess of cold antagonist is added during the washing^{4,7}. In the absence of excess cold antagonist, binding seems to be virtually irreversible. The amount of specifically bound (●) ^{125}I -HYP is the calculated difference between total (○) and nonspecific binding (□).

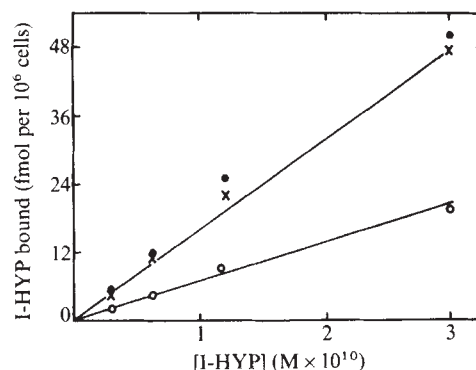


Fig. 2 ^{125}I -HYP binding to L8 muscle cells in the absence of phentolamine. About 2×10^6 cells per 6-cm plate were incubated with 2 ml of PBS containing various concentrations of ^{125}I -HYP, in the absence of phentolamine, without propranolol (●) and in the presence of 2.5×10^{-5} M (○) or 10^{-7} M (x) (–)propranolol and assayed for binding as described in Fig. 1.

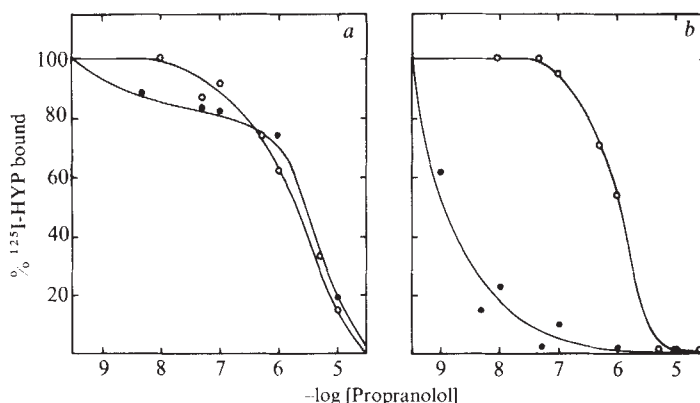
We have recently devised a method to measure the number of β adrenoreceptors on intact C6 glioma cells². That study demonstrated that tissue culture cells bind large amounts of ^{125}I -hydroxybenzylpindolol (^{125}I -HYP) in a nonspecific manner. This nonspecific binding can be largely eliminated by carrying out the binding assays in the presence of high concentrations of phentolamine as originally observed by Sporn and Molinoff³ in membranes from rat brain. Figure 1 shows that 5×10^{-5} M phentolamine displaces 75% of the nonspecifically bound ^{125}I -HYP but does not modify the specifically bound ligand.

In addition, the concentration of cold competing antagonist or agonist used to measure the nonspecific binding must be chosen according to its dissociation constant and in relation to the concentration of radioactive ligand. In our study, 10^{-7} M (–)propranolol was a satisfactory concentration ($100 \times K_D$). Higher concentrations are not necessary to displace ^{125}I -HYP bound to β receptors and such high concentrations can displace ^{125}I -HYP bound nonspecifically.

Figure 2 shows the difference in two nonspecific binding curves obtained on L8 muscle cells in the presence of two different concentrations of (–)propranolol, 2.5×10^{-5} M and 10^{-7} M in the absence of phentolamine. The difference between these two curves represents displacement of nonspecifically bound radioactive ligand.

This conclusion is supported by the results shown in Fig. 3. 10^{-7} M (–)propranolol, the pharmacologically active optical

Fig. 3 Inhibition of ^{125}I -HYP binding to L8 muscle cells by propranolol. Binding was carried out as described in Fig. 1, in the absence (a) or presence (b) of 5×10^{-5} M phentolamine, with (+)propranolol (○) or (–)propranolol (●). 100% and 0% binding refer, respectively, in a to 100,000 and 30,000 c.p.m. and in b to 40,000 and 22,000 c.p.m. These experiments were done in parallel on the same number of cells and the results can thus be compared quantitatively as well as qualitatively.



isomer, provokes nearly maximal displacement from stereospecific sites. The reduction in binding observed in the absence of phentolamine with concentrations of propranolol higher than 10^{-7} M is clearly non-stereospecific and thus represents displacement from nonspecific sites. Figure 3b, in which most of the nonspecific ^{125}I -HYP bound has been removed in the presence of phentolamine, strikingly illustrates the stereospecific displacement from β receptors. Atlas *et al.* carried out their assays with similar concentrations of radioactive ligand but in the absence of phentolamine. In these conditions, their concentration of ($\pm$)propranolol, 5×10^{-5} M, was two orders of magnitude too high and displaced ^{125}I -HYP bound to sites unrelated to β -adrenergic receptors. It should be noted that the relative amount of nonspecifically bound ^{125}I -HYP is highly variable among different cell lines. In turkey erythrocytes,

adrenoreceptors on rat muscle cells of the L8 or L6 line does not single them out for receptor purification, it is, however, sufficient to analyse β -adrenoreceptor distribution during muscle cell fusion.

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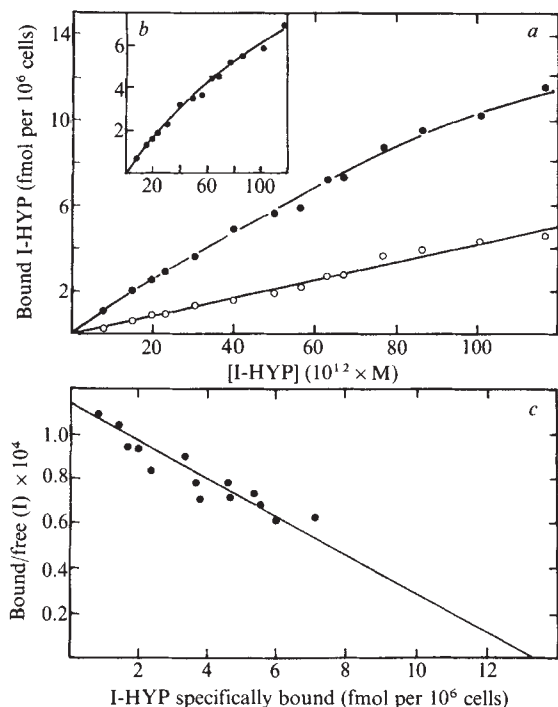


Fig. 4 Binding of ^{125}I -HYP to intact L8 muscle cells in the presence of phentolamine. Assays were carried out in standard conditions. 3×10^6 cells per plate were incubated with 5×10^{-5} M phentolamine and various concentrations of ^{125}I -HYP. In a, the total amount of ^{125}I -HYP bound (●) and the nonspecifically bound ligand remaining in the presence of 10^{-7} M (–)propranolol (○) are plotted as a function of the ^{125}I -HYP. Specific binding, defined as the difference between the total and the nonspecific binding, is plotted in b. The saturation value (13.2 fmol per 10^6 cells or 7,950 receptors per cell) and the K_D (1.2×10^{-10} M) has been calculated using a linear regression curve (coefficient of correlation = 0.92) obtained with the Scatchard plot represented in c.

nonspecific binding is much lower and thus the use of 10^{-7} M or 5×10^{-5} M ($\pm$)propranolol would not change the amount of displaced ^{125}I -HYP in such a dramatic fashion. In Fig. 4, the specific binding of ^{125}I -HYP to β adrenoreceptors has been measured in the presence of phentolamine using 10^{-7} M (–)propranolol as the competing ligand. Scatchard analysis of these data shows that L8 muscle cells have $7,900 \pm 1,600$ specific β adrenoreceptors per cell. This number is not significantly different from that obtained in C6 glioma cells^{2,4,5}. The use of 2.5×10^{-5} M (–)propranolol (Fig. 2) without phentolamine gives an erroneously high number of receptors per cell, thus explaining the figure given by Atlas *et al.*

Our results demonstrate the importance of the choice of experimental conditions in the determination of the true number of stereospecific β receptors. Although the number of β

ATLAS, HANSKI AND LEVITZKI REPLY—Our reply will be divided into two parts: first, the validation of our results¹, and second, the demonstration that the methodology used by Pochet and Schmitt is inadequate.

To consider our first point. The difference between the binding curve of ^{125}I -($\pm$)hydroxybenzylpindolol (HYP) in the absence and in the presence of 5.0×10^{-5} M ($\pm$)propranolol monitors the reliable number of β adrenoreceptors in intact cells as well as in membrane fragments prepared from these cells. Figure 1 shows Scatchard plots of the binding of ^{125}I -($\pm$)-HYP to L6P cells removed from the growth plate by EDTA as compared with L6P cells removed by using trypsin. It can be seen that trypsin causes a five- to sixfold decrease in the number of β adrenoreceptors. The destruction of receptors by trypsin is well documented for several hormone receptors including β adrenoreceptors. In contrast to the effect of trypsin on the number of β adrenoreceptors detected, no change was observed in the number of ^{125}I -($\pm$)HYP binding sites by changing the fluidity of the cell membrane. A large change in the fluidity of the turkey erythrocyte membrane has no effect on the extent of the 'nonspecific' or specific ^{125}I -($\pm$)HYP binding. Table 1 summarises results demonstrating that the ratio between cholesterol and phospholipids has no influence on the binding of ^{125}I -($\pm$)HYP to intact turkey erythrocytes or to membrane fragments prepared from these cells. Further details on this particular point are given elsewhere². The fluidisation of turkey erythrocyte membranes using free fatty acids also has no influence on the number of specific ^{125}I -($\pm$)HYP binding sites on the turkey erythrocyte or on the affinity of these receptors to the β blocker¹¹.

The number of β adrenoreceptors on a turkey erythrocyte, as measured by ^{125}I -($\pm$)HYP, is identical to the number found by the ^3H -($\pm$)propranolol technique introduced by us³⁻⁵. Furthermore, the measurement of the content of β -adrenergic receptors using ^3H -($\pm$)isoprenaline again gives similar results³. It is worth noting that the difference between ^{125}I -($\pm$)HYP gives linear Scatchard plots, thus indicating that a single class of ^{125}I -($\pm$)HYP binding sites is being monitored. If Pochet and Schmitt were correct in their assertion that ($\pm$)propranolol displaced both specific and nonspecific ^{125}I -($\pm$)HYP, one would

Table 1 Effect of cholesterol enrichment and of cholesterol depletion on the β -adrenergic system

Treatment	Cholesterol to phospholipid ratio	^{125}I -HYP binding (pmol per mg)	^{125}I -HYP receptor dissociation constant
No treatment (control)	1.0–1.2	0.55 ± 0.05	$(6.8 \pm 0.5) \times 10^{-11}$
Cholesterol depleted	0.5–0.6	0.53 ± 0.05	$(7.5 \pm 0.05) \times 10^{-11}$
Cholesterol enriched	1.45–1.55	0.54 ± 0.05	$(6.9 \pm 0.5) \times 10^{-11}$

observe at least two classes of ^{125}I -($\pm$)HYP binding sites which should have given curvilinear Scatchard plots. In our binding studies on intact cells of different types, L6P cells, HeLa cells, turkey erythrocytes and turkey reticulocytes, we have consistently found a single class of ^{125}I -($\pm$)HYP binding sites^{2,6}. It should be noted that the successful use of ^{125}I -($\pm$)HYP in the determination of the number of β adrenoreceptors on intact cells was first introduced by Aurbach *et al.*⁷ and has since been adopted by several laboratories, including our own. Furthermore, the methodology used by us was originally introduced by Aurbach *et al.*⁸ and was demonstrated to be an equilibrium technique^{8,9}. Maguire *et al.*⁹ have also used propranolol (1.0×10^{-5} M (-)propranolol) for the displacement of the non-specific ^{125}I -($\pm$)HYP bound, obtaining reliable and consistent binding data.

Schmitt and Pochet are correct in asserting that concentrations lower than 5.0×10^{-5} M ($\pm$)propranolol should be sufficient to displace the specific ^{125}I -($\pm$)HYP bound. Indeed, 2.0×10^{-7} M, 2.0×10^{-6} M and 2.0×10^{-5} M ($\pm$)propranolol displace bound ^{125}I -($\pm$)HYP to a similar extent. Interestingly, using the usual methodology as described^{1,2,6–10}, we found that inclusion of 2.5×10^{-5} M phentolamine in the binding mixture reduces the nonspecific binding of ^{125}I -($\pm$)HYP by 10–15% but does not modify the pattern of ^{125}I -($\pm$)HYP to the specific sites. As the effect of phentolamine is marginal (see also below), we find no need to use it. These results are similar to those reported by Brown *et al.* for turkey erythrocytes¹⁰.

On our second point, the methodology used by Pochet and Schmitt suffers from several flaws which cast serious doubts on the validity of their experimental approach. (1) What the authors call 'nonspecific' ^{125}I -($\pm$)HYP binding is removed by six

washings, 10 min each, at 37 °C. The methodology used by the authors is cited in their manuscript (their ref. 2). As the half life of ^{125}I -($\pm$)HYP on the β adrenoreceptors is $t_{1/2} = 7.2$ min at 30 °C (ref. 7) and $t_{1/2} = 2.57$ min at 35 °C (ref. 8), it is clear that most of the specific ^{125}I -($\pm$)HYP bound to the β adrenoreceptors is washed out. Therefore, the methodology used is not an equilibrium technique and cannot give reliable data. (2) The cells are removed by trypsin. As we have shown (Fig. 1 and ref. 6), the β adrenoreceptors on L6P cells are destroyed in these conditions. This was the reason EDTA^{1,6} was used instead. No evidence is provided by the authors that the 'recovery' period results in the resynthesis of the β adrenoreceptors. (3) The authors use phentolamine to displace nonspecific ^{125}I -($\pm$)HYP binding. At least in one case (rat hypothalamus membranes) phentolamine was found to displace ^{125}I -($\pm$)HYP from β adrenoreceptors¹². It should be noted that phentolamine, at the concentration range of 1.0×10^{-5} to 1.0×10^{-4} M, can block the isoprenaline-dependent adenylate cyclase activation in neuroblastoma-glyoma hybrids (unpublished data). In our experiments, phentolamine had an insignificant effect. This large variability in phentolamine action on different cells convinces us that its use in binding studies is dispensable. (4) As to the quality of the binding curves presented by Pochet and Schmitt, their Fig. 2 does not show any of the curvature which should be observed if the binding of ^{125}I -($\pm$)HYP has a saturable component. The data presented in their Fig. 4 do not demonstrate that saturation has been achieved. The data can be easily fitted by a straight line. A replot of the raw data in their Fig. 4a, according to Scatchard, shows that only 43% saturation has been achieved. If one believes that the remaining 57% behave in a similar fashion, the maximal number of receptors should be 9,650 receptors ($K_D = 1.6 \times 10^{-10}$ M) per cell. In view of the methodology used by the authors, we believe that this number underestimates the true number of β receptors by about 80–90%. (5) The authors used L8 cells, whereas we used L6P cells.

Note added in proof: Recently the density of β -receptors was measured on L84 rat skeletal myogenic muscle cells, using an ultrastructural probe for the receptor. Using this technique receptor density was found¹³ to be 71 ± 19 receptors per μm^2 . That is, 50,000 to 80,000 receptors per cell—similar to the closely related L6 cells.

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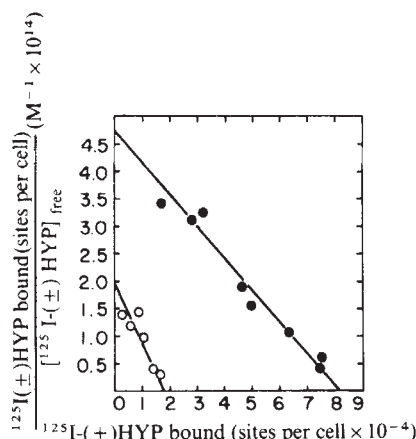


Fig. 1 ●, L6P cells removed by EDTA treatment. Experimental details are given elsewhere^{1,6}. ○, L6P cells were removed by treatment with trypsin. The growth medium was removed from the tissue culture bottle and the cells were incubated with 50 ml 130 mM saline, containing 20 mM Tris-HCl buffer, pH 7.4, and 500 $\mu\text{g ml}^{-1}$ trypsin, for 30 min at 37 °C. The cells were detached from the bottle, centrifuged at 500 g, and washed twice with trypsin-free saline containing buffer. The binding procedure was the same as described elsewhere^{1,2,6}.

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Minor differences between nucleotide sequences of mild and severe strains of potato spindle tuber viroid

VIROIDS are the smallest replicating pathogenic agents known¹. Composed entirely of RNA, they have genome sizes in the range of 330–380 nucleotides², 10 times smaller than the smallest bacteriophage of *Escherichia coli*³. Although there is no evidence for the existence of a protective protein coat for viroids, they infect a wide variety of plants and in many produce

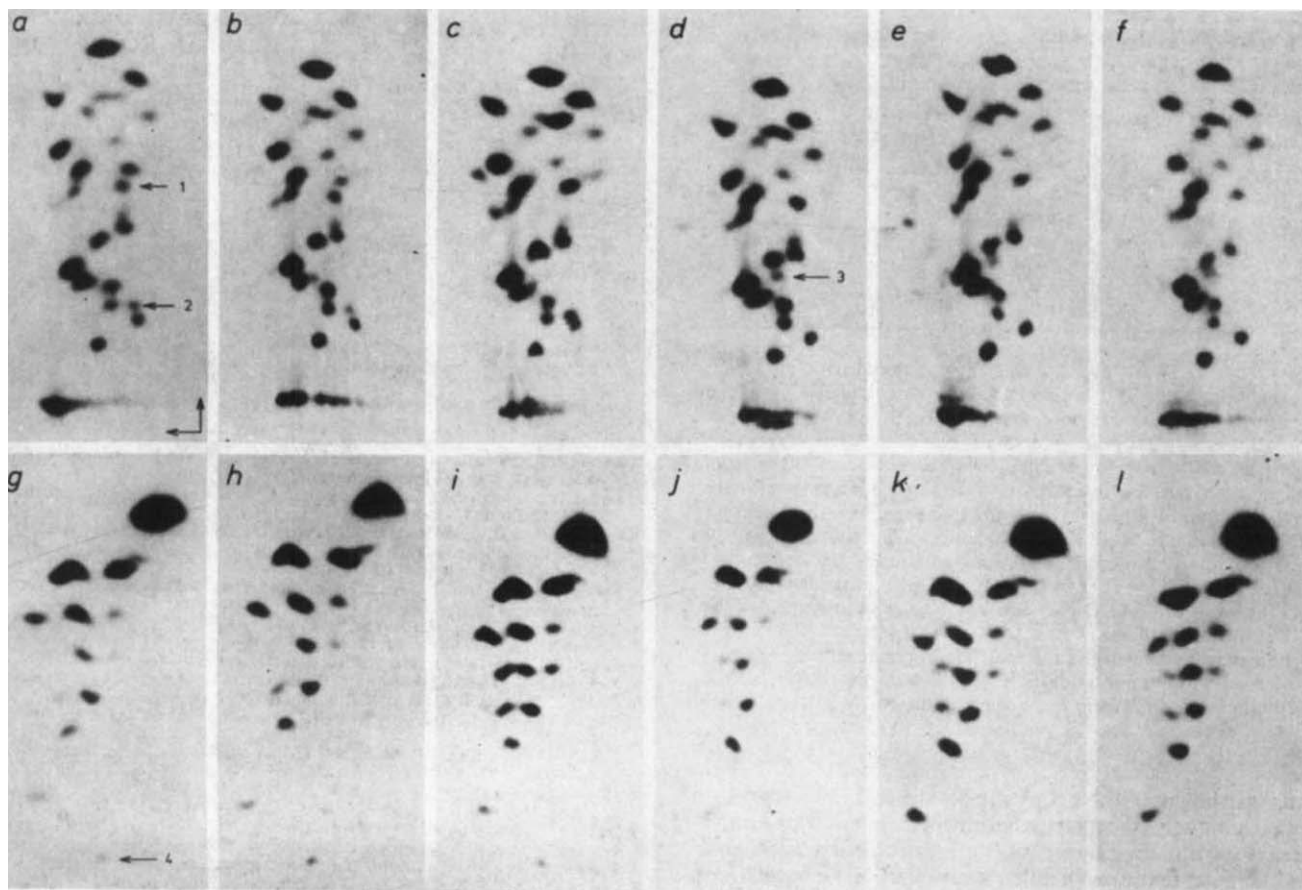


Fig. 1 Ribonuclease T1 and pancreatic ribonuclease fingerprints of two severe, one intermediate and three mild strains of PSTV. 1×10^6 c.p.m. aliquots of each ^{125}I -labelled viroid were dried down in silicone-treated test tubes and digested with $2 \mu\text{l}$ of either ribonuclease T1 (1 mg ml^{-1} for 40 min at 37°C) or pancreatic ribonuclease (1 mg ml^{-1} for 30 min at 37°C) in 0.01 M Tris-HCl, $\text{pH } 7.6$, 0.001 M EDTA in the presence of $10 \mu\text{g}$ of tRNA carrier as described previously^{12,20}. The origin of each autoradiograph is at the lower right. The first dimension (right to left) consists of high-voltage electrophoresis on cellulose acetate at $\text{pH } 3.5$, while the second consists of ascending RNA homochromatography on thin layers of DEAE-cellulose²⁰. Fingerprints were exposed to Dupont Cronex 2 X-ray film at -70°C in 14 in. $\times$ 17 in. Halsey-Radelin cassettes in the presence of two Dupont Lightning-plus intensifying screens. Fingerprints of 6 of the 11 PSTV isolates studied are shown. In each case, the ribonuclease T1 fingerprint is placed vertically above the pancreatic ribonuclease fingerprint; a and g, severe isolate, PS; b and h, severe isolate, SE; c and i, intermediate isolate, PD; d and j, mild isolate, PM; e and k, mild isolate, 6; f and l, mild isolate, 12. For an account of the source of each of these isolates, see the footnotes to Table 1. The numbered arrows in panels a, d and g indicate spots which are diagnostic for one or another strain of PSTV as discussed in the text and summarised in Table 1.

severe disease symptoms^{1,4}. The molecular mechanisms by which viroids replicate and interact with their hosts are not yet understood. In its most severe form, the disease^{5,6} caused by potato spindle tuber viroid (PSTV) causes general stunting of potato plant growth, deformity of the upper foliage, and production of disfigured potatoes⁵. Mild strains of PSTV which produce barely detectable symptoms have also been isolated⁷. Furthermore, plants infected by mild strains are somehow protected from developing symptoms following subsequent inoculation with severe strains^{8,9}. This biological protection phenomenon has been called cross-protection⁷. As the only known component of viroids is RNA, mild and severe strains must differ in nucleotide sequence. The study described here aimed to determine the extent of this difference.

Four independently maintained severe, one intermediate, and six mild isolates of PSTV were propagated in Rutgers tomato plants, extracted and purified as described elsewhere¹⁰, labelled *in vitro* with ^{125}I (ref. 11), and subjected to two-dimensional RNA fingerprint analysis¹¹⁻¹³. Because the fingerprints obtained showed evidence of variable quantities of host RNA contaminants (data not shown), the ^{125}I -labelled viroid preparations were repurified by electrophoresis in 5% polyacrylamide gels¹¹ and fingerprinted once again.

Ribonuclease T1 and pancreatic ribonuclease fingerprints of two severe, one intermediate, and three mild PSTV isolates are shown in Fig. 1. The other two severe and three mild isolates

studied did not differ significantly from those illustrated. Visual inspection reveals that all six ribonuclease T1 fingerprints closely resemble each other, as do all six pancreatic ribonuclease fingerprints. However, a few differences are evident. The four spots found to be diagnostic for the various strains are indicated in Fig. 1 by numbered arrows. The four severe strains have spots 1, 2 and 4 but lack spot 3 (Fig. 1 a, b, g, h). The intermediate strain has spots 2 and 4 but lacks spots 1 and 3 (Fig. 1 c, i). The six mild strains have spot 3 but lack spots 1, 2 and 4 (Fig. 1 d, e, f, j, k, l).

Secondary enzymatic digestion of all oligonucleotides from the fingerprints shown in Fig. 1 a, d, g, j followed by identification of cleavage products (as described in refs 12, 13) revealed only two further differences between the mild and severe strains of PSTV. The pancreatic ribonuclease fingerprint of mild PSTV contained the hexamer AAGGAC and the tetramer GAGC, neither of which can be detected by visual examination as all strains of PSTV analysed have two other hexanucleotides, GAGAAC and GGAAAC, which co-migrate with AAGGAC; and AGGC which co-migrates with GAGC. As the sequence of spot 4 is AAAAAAGGAC, there is the attractive possibility that the new hexanucleotide AAGGAC found in mild strains is the result of the replacement of the fourth A-residue of spot 4 by a pyrimidine. These sequences were determined as described previously^{12,14-17}. The intermediate strain (Fig. 1 i) lacks AAGGAC and contains GAGC.

Table 1 Oligonucleotide differences observed among severe, intermediate and mild strains of PSTV

Oligonucleotide description*	Severe	PSTV strain† Intermediate	Mild
spot 1	+‡	—	—
spot 2	+	+	—
spot 4	+	+	—
spot 3	—	—	+
AAGGAC	—	—	+
GAGC	—	+	+

* Spots 1, 2, 3 and 4 are those indicated by numbered arrows in Fig. 1.

† Eleven isolates of PSTV were selected for these studies. One of the four severe isolates (A) was obtained from the Schultz Collection of Potato Pathogens at Beltsville, Maryland. The other three severe isolates (PS, D and SE) were obtained over the course of 30 yr from naturally infected potatoes in field plots of the Cornell University Potato Breeding Program by Dr Karl H. Fernow. This was also the source of five of the six mild isolates (PM, 6, 7, 10 and 11). The sixth mild isolate (12) was recovered in autumn 1977 from a naturally infected potato plant at Riverhead, New York by Dr Fernow. The intermediate isolate, PD, was the gift of Dr T. O. Diener and was the one we used in previous RNA fingerprinting studies^{11,13,16}.

‡ + indicates the presence of the specified oligonucleotide in fingerprints of the PSTV strain in question. — indicates the absence of the specified oligonucleotide from fingerprints of the PSTV strain.

Thus, as shown in Table 1, while severe and mild strains of PSTV are uniquely characterised by the presence and absence of particular spots in their fingerprints, the intermediate strain has no such singular features. Detailed study of all data derived from oligonucleotide secondary analysis also permits us to estimate that mild and severe strains differ in 2–10 of their nucleotide residues (E.D. and H.D.R., unpublished observations).

These results demonstrate that the marked contrast between severe and mild symptoms must be explained on the basis of relatively few specific nucleotide sequence differences. As noted above, one of the sequence differences seems to occur at the fourth residue of the decanucleotide AAAAAAGGAC (spot 4; see Fig. 1 and Table 1). This can be identified as position 120 within the sequence of an intermediate strain¹⁰ of PSTV (refs 18, 19). Further sequence analysis will be required before the exact positions of the other oligonucleotides associated with the mild-severe difference can be assigned. Such studies should also allow us to determine whether the residues important for the pathogenic properties are clustered in a small region of the viroid genome or are widely separated.

Although mild and severe strains of PSTV have very similar nucleotide sequences, additional studies indicate that such extensive sequence homology is not a prerequisite for cross-protection. Although comparative RNA fingerprint analysis of PSTV, citrus exocortis viroid (CEV) and chrysanthemum stunt viroid (CSV) have demonstrated that the sequences of these three viroids differ extensively from each other^{11,13,16}, biological studies have recently shown⁹ that mild strains of PSTV cross-protect, not only against severe strains of PSTV, but also against CEV and CSV.

If cross-protection and disease symptom formation are determined by common features within a portion of the viroid genome, further studies should allow us to identify the regions and assess the influence of RNA sequence and structure on these biological properties.

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Selecting *Rhizobium* for acid, infertile soils of the tropics

AN increase in beef cattle production on the 350 million hectares of acid, infertile savannahs of tropical South America would have a major impact on world food production. Production in these regions is limited by the inadequate nutritional value of the native vegetation, and legume-based pastures are now being introduced¹ to raise the protein content of available forage. However, to obtain full benefit from biological nitrogen fixation through nodulated legumes it is necessary that the introduced legume form an effective symbiosis with *Rhizobium*. The low pH, limited availability of phosphorus, and very high levels of aluminium and manganese in these soils, are likely to affect nodulation and nitrogen fixation. It is therefore essential that strains of *Rhizobium* adapted to acid conditions are used. Alkali production by rhizobia from tropical legumes and the consequent rise in pH of test media has made the development of a simple test to select such strains difficult². We report here that interference from alkali production can be eliminated by minor changes in the screening medium. This finding challenges the view that alkali production is a distinguishing characteristic with ecological significance³, and is also a starting point for detailed study of the effects of acidity and related factors on growth of *Rhizobium*.

In an evaluation of 50 *Rhizobium* strains (from soils ranging from pH 4.5 to 9.0) isolated from nodules obtained from *Stylosanthes capitata*, *S. guianensis* and *S. hamata* we observed marked differences between strains in their ability to grow in two yeast-mannitol (YM) media differing in pH. Strains fell into one of three categories: 19 grew only in the standard YM medium of pH 6.8–7.0; 2 grew only in an acidified medium (adjusted to pH

4.5 with 0.1 M HCl); and 29 grew in both media. However, media which were acid initially had a final pH in the range 7 to 8 due to an alkalinising effect which is claimed to be a differentiating characteristic of slow-growing tropical rhizobia³. Therefore, growth could indicate either genuine ability to multiply at low pH or capacity to progressively increase the pH of the medium to an adequate level for growth. A stable acid medium is needed that selects specifically for the former character as strains which grow as a result of influencing their environment during the test are unlikely to be successful in more highly buffered acid soils.

A range of media differing in composition and pH was evaluated as substrate for the growth of two strains of contrasting origins (acid soil, Llanos Orientales, Colombia, pH 4.5 compared with alkali soil, near Kingston, Jamaica, pH 8.5). The strain of alkali soil origin grew in media of initial pH 7 and pH 8.5 but not in a medium of pH 4.5. The strain of acid soil origin grew best in the medium of pH 4.5, made little growth at pH 7 and even less at pH 8.5. Growth of the acid soil origin strain was equally good irrespective of whether the medium was acidified using hydrochloric acid, sulphuric acid or citric acid. It also grew satisfactorily in media incorporating other acidity stresses, that is, low P (10 μ M), high Al (50 μ M), low Ca (50 μ M), and high Mn (200 μ M). Growth of *Rhizobium* in acidified media incorporating mannitol, the standard carbon source recommended for culturing rhizobia, raised the pH from 4.5 to approximately 7.5. However, in acidified media in which arabinose, or alternatively galactose, was substituted for mannitol, the pH decreased slightly (to 3.8) yet growth (turbidity/absorbance) was essentially the same as in media incorporating mannitol. Previous attempts to stabilise culture media at acid pH for growth of *Rhizobium* by using buffers have proved unsatisfactory⁴. Other reports concerning optimal pH for growth of slow-growing rhizobia^{5,6} and utilisation of carbohydrates⁷ have referred to initial pH values. Final pH values have been reported for growth in a range of carbon sources but from an initial pH of approximately 7 (ref. 8).

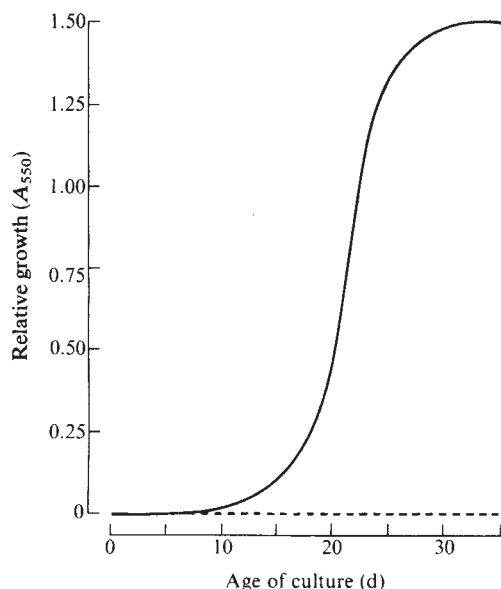


Fig. 1 Relative growth of *Rhizobium* strains CIAT 1460 (solid line), originating from an acid soil (pH 4.5), and CB 2126 (dashed line), from an alkali soil (pH 8.5), in a defined, acid medium (initial pH 4.2).

Growth of *Rhizobium* was quantified for a defined basal mineral salts liquid medium (Table 1) which was one of the media used previously. Cultures were incubated at 25–28 °C and aerated by shaking. Growth, both as optical absorbance at 550 nm and as viable cell number, and pH were determined over a period of 33 d in media which differed only in their carbon source (mannitol or arabinose). In both media, the acid soil origin strain CIAT 1460 showed exponential growth (Fig. 1),

Table 1 Liquid medium for selecting acid-adapted strains of *Rhizobium*

Addition	Quantity added
KH ₂ PO ₄	68 mg
K ₂ HPO ₄	87 mg
CaCl ₂ ·2H ₂ O	39.4 mg
MgSO ₄ ·7H ₂ O	74 mg
Fe ³⁺ +Na EDTA	36.8 mg
MnCl ₂ ·4H ₂ O	0.49 mg
ZnSO ₄ ·7H ₂ O	0.29 mg
CuCl ₂ ·2H ₂ O	43 μ g
Na ₂ MoO ₄ ·2H ₂ O	12 μ g
CoCl ₂ ·6H ₂ O	1.2 μ g
Sodium glutamate	220 mg
*Thiamine	100 μ g
*Biotin	250 μ g
Arabinose	10 g
Distilled water	to one litre

Acidified with 0.1 M HCl (before autoclaving).

* Filter sterilised and added after autoclaving the medium.

increasing from an inoculum of 7×10^3 to 1×10^9 viable cells per ml whereas the number of viable cells per ml of alkali soil origin strain CB 2126 diminished from 4×10^4 to 2×10^2 . Growth of the acid soil strain in mannitol medium was associated with a substantial pH rise (Fig. 2); in arabinose medium, however, the pH declined from 4.2 to 3.9. Growth (viable cell number) of CIAT 1460 did not differ significantly in the two media.

The finding that some strains of *Rhizobium* grow much better at pH 4.5 than in neutral media is contrary to the commonly accepted view that *Rhizobium* has a pH growth optimum of ~7 (refs 9, 10). It also helps explain why attempts to isolate *Rhizobium* from nodules of *Soylosanthus* (especially *S. capitata*) growing in soils at pH 4.5–5.5 have been unsuccessful—the routine isolation medium has a pH 6.8–7.0. In addition it suggests that the standard practice of searching for acid stress tolerant *Rhizobium* in existing collections of strains that were originally isolated onto routine non-stress media may not be successful.

Now that this medium is available it will be possible to test the effects of characteristic acid-soil conditions, such as high aluminium content, on *Rhizobium*. Comparison of strains in the early phase of their growth before a change in the pH of the

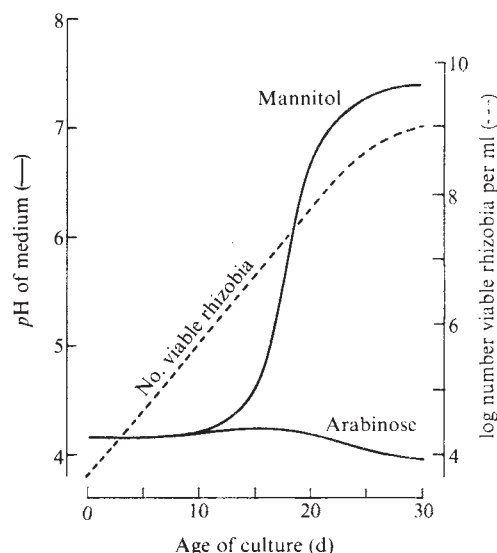


Fig. 2 The pH changes during culture of *Rhizobium* strain CIAT 1460 in defined liquid media containing either the standard carbohydrate source mannitol, or an alternative source, arabinose. Rhizobia multiplied at the same rate in both media from an initial viable cell number of 7×10^3 to 1×10^9 cells per ml.

medium can be detected⁷ has the disadvantages that strain differences have not become fully pronounced and that the growth must be estimated by the extremely tedious viable count method. Availability of an acid medium in which the pH does not rise permits a simple test in which attainment of visual turbidity indicates ability to grow at low pH.

Finally, our demonstration that alkali production by slow-growing, tropical-type rhizobia is dependent on the carbohydrate of the culture medium reopens the question of whether these microorganisms actually produce alkali in the rhizosphere of their host and whether an ecological role should have been attributed to the process³.

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Inactivation of restriction endonuclease *Bam*Nx after infection with phage ϕ NR2

A HOST-CONTROLLED restriction-modification system¹⁻³ is a means of protection by a host against attacks of bacteriophages. Recently, coliphage T3 and T7 have been found to overcome⁴⁻⁷ the host-controlled restriction-modification systems of their host strains *Escherichia coli* B and K, but details of the mechanism are not well understood at present. We have found that a *Bacillus subtilis* phage ϕ NR2rH, a mutant of ϕ NR2 (ref. 8), also has the ability to overcome the restriction-modification system of *B. amyloliquefaciens* strain N, and detected a *Bam*Nx inhibitor in cells infected with ϕ NR2rH.

Shibata and Ando found two different restriction endonucleases⁹⁻¹¹, *Bam*NI (*Bam*NI cleaved DNA in the same nucleotide sequence as *Bam*HI (refs 12, 13)) and *Bam*Nx in the strain N. *Bam*Nx is a typical type II restriction endonuclease. The phage ϕ NR2 and its host *B. subtilis* PS9 (derivative of *B. subtilis* Marburg strain sensitive to phage SP10 and ϕ NR2) were isolated by Yajima *et al.*^{8,14}. Since ϕ NR2 DNA is completely insensitive to *Bam*NI, the phage ϕ NR2 can form plaques normally on both PS9 and *B. amyloliquefaciens* strain H. ϕ NR2 (grown on PS9) however, forms plaques on strain N at a frequency of about 4×10^{-4} . Initially we thought that the phage ϕ NR2 was restricted by *Bam*Nx of strain N and that the survived phages would be modified. One clone, isolated from the plaques on strain N, was no longer restricted by this strain. Nevertheless, the DNA, prepared from phage particles grown on strain N, was

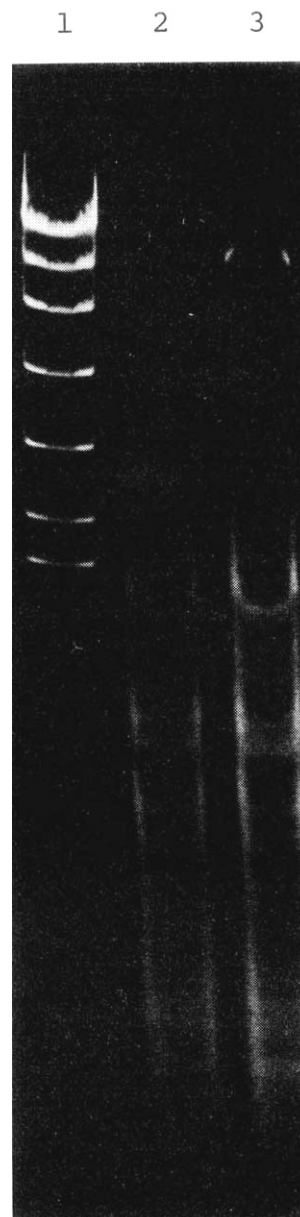


Fig. 1 Cleavage of DNAs of ϕ NR2 (grown on PS9) and ϕ NR2rH (grown on strain N) with endonuclease *Bam*Nx. Each DNA (1 μ g) was reacted with *Bam*Nx in 20 μ l of 20 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM EDTA, 15 mM MgCl₂ and 5 mM 2-mercaptoethanol at 37 °C for 60 min. Cleaved DNAs were adjusted to 8% glycerol and 0.02% bromophenol blue and heated for 2 min at 60 °C. The samples were applied to 0.8% Agarose (Seakem) slab gel (15 $\times$ 15 $\times$ 0.3 cm). Electrophoresis was carried out at room temperature for 2.5 h at a constant current of 90 mA. The gel was stained with ethidium bromide and photographed with short-wave ultraviolet light. Löening's buffer¹⁵ (36 mM Tris-HCl, pH 7.7, 30 mM NaH₂PO₄, 1 mM EDTA) was used for electrophoresis and staining¹⁶. 1, *Bam*NI fragments of *B. subtilis*-phage ϕ 11C3a DNA as standard markers (see Fig. 3 in detail); 2, ϕ NR2 (grown on PS9) DNA cleaved with *Bam*Nx; 3, ϕ NR2rH (grown on strain N) DNA cleaved with *Bam*Nx.

still cleaved by *Bam*Nx (Fig. 1). These results suggested that this mutant has the ability to overcome the host-controlled restriction-modification system of strain N. The mutant was designated as ϕ NR2rH because it formed rapid lysis-type plaques on strain H. ϕ NR2rH was inactivated by anti- ϕ NR2 serum, and its DNA gave the same *Eco*RI cleavage pattern as that of ϕ NR2 DNA (data not shown), indicating that ϕ NR2rH is a spontaneous mutant of ϕ NR2.

ϕ NR2rH was not restricted by strain N, whereas its DNA was cleaved by *Bam*Nx from strain N, *in vitro*. We therefore

examined whether or not the activity of *Bam*Nx still remains in N cells infected with ϕ NR2rH. Crude extract was prepared from phage-infected cells by the following procedure. Cells of strain N were infected with ϕ NR2rH at a multiplicity of five when the number of cells became approximately $1.5 \times 10^8 \text{ ml}^{-1}$ in L-broth at 37°C with shaking. The infected cells were collected 15 min after infection and stored at -20°C . These cells were then thawed, suspended in 20 mM Tris-HCl buffer (pH 7.5) containing 5 mM MgCl_2 , 0.2 mM EDTA and 5 mM 2-mercaptoethanol, and sonicated for 15 min in an ice-water bath. After removal of the cell debris by centrifugation at $80,000g$ for 90 min at 4°C , 5% streptomycin sulphate solution was added gradually, to give a final concentration of 1%. The suspension was centrifuged and the supernatant was dialysed at 4°C overnight against the 20 mM Tris-HCl buffer described above. The precipitate which appeared during dialysis was removed by low-speed centrifugation. The final supernatant was the crude extract used in this study. Crude extracts were also prepared from ϕ NR2-infected and uninfected N cells, as was the ϕ NR2rH-infected cell extract. We could detect *Bam*Nx activity

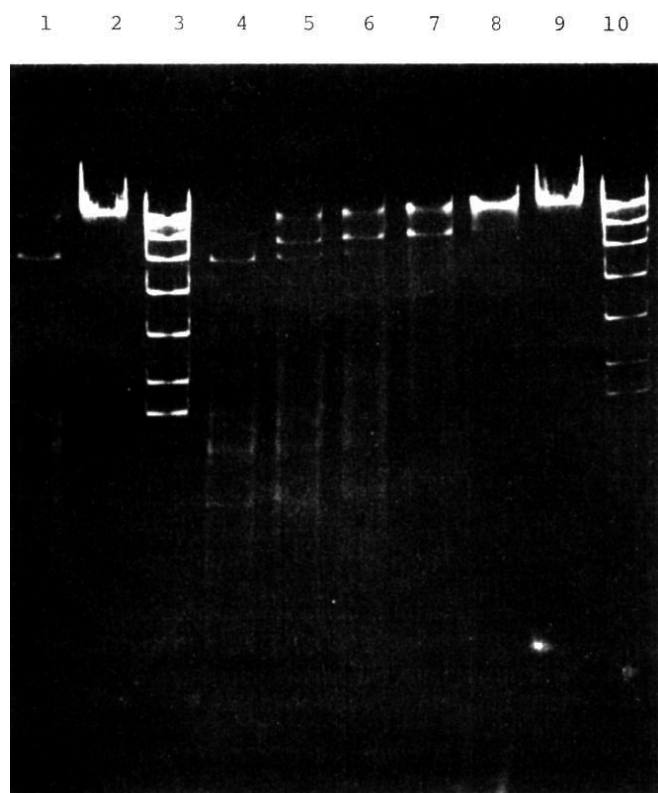


Fig. 2 Detection of *Bam*Nx inhibitor in the crude extract from ϕ NR2rH-infected N cells using ϕ NR2rH DNA as the substrate of *Bam*Nx. Purified *Bam*Nx was preincubated with various amounts of the crude extract, prepared as described in the text, in 50 μl of 20 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM EDTA, 15 mM MgCl_2 , 5 mM 2-mercaptoethanol at 37°C for 15 min. After this treatment, 2.5 μg of ϕ NR2rH DNA extracted from purified phage particles grown on strain N were added, incubated for 60 min at 37°C and 30 μl of each sample were applied to electrophoresis for the analysis of the remaining *Bam*Nx activity. Electrophoresis was carried out as described in Fig. 1. *Bam*NI fragments of *B. subtilis*-phage ρ 11C3a DNA were used as standard markers (see Fig. 3 in detail). 1, Remaining activity of *Bam*Nx after the preincubation with 25 μl of the crude extract (about 7 mg protein per ml) from uninfected N cells; 2, untreated ϕ NR2rH DNA; 3, *Bam*NI fragments of ρ 11C3a DNA; 4–8, inactivation of *Bam*Nx by the preincubation with the crude extract from ϕ NR2rH-infected N cells. Purified *Bam*Nx was preincubated with 0 μl (4), 1 μl (5), 3 μl (6), 10 μl (7) and 25 μl (8) of the crude extract from ϕ NR2rH-infected N cells (about 8 mg protein per ml). 9, untreated ϕ NR2rH DNA; 10, *Bam*NI fragments of ρ 11C3a DNA.

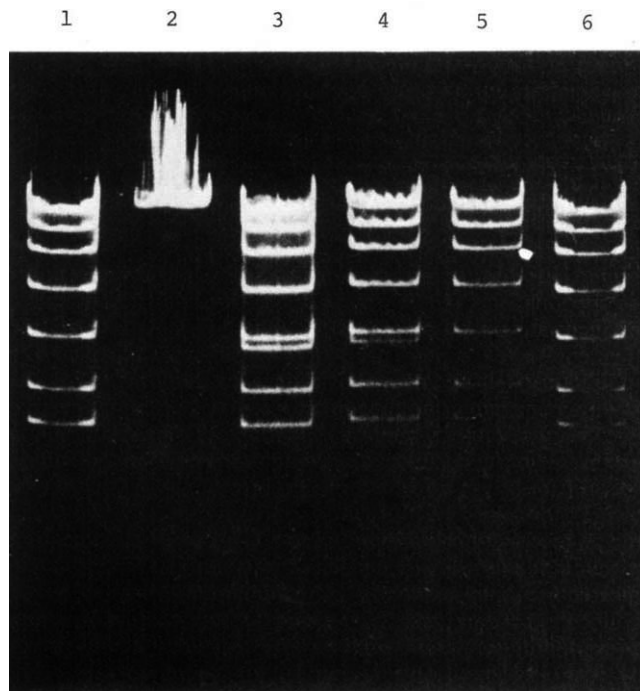


Fig. 3 Detection of *Bam*Nx inhibitor using ρ 11C3a DNA as the substrate of *Bam*Nx. The same crude extracts as described in Fig. 2 were used. *B. subtilis*-phage ρ 11C3a DNA generates 10 fragments by *Bam*NI (or *Bam*HI) cleavage, and 2 fragments by *Bam*Nx cleavage (Kawamura and Ito, in preparation). *Bam*NI fragments were designated *Bam*NI A–J in order of decreasing size. *Bam*Nx cleaves *Bam*NI A fragment and a new DNA band appears between the *Bam*NI E and F fragments by Agarose gel electrophoresis after cleavage with both *Bam*NI and *Bam*Nx. It is thus possible to determine the activity of *Bam*NI and/or *Bam*Nx using this DNA as substrate. The preincubation of purified *Bam*Nx with the crude extract was performed in the same conditions as described in Fig. 2. Electrophoresis was carried out as described in Fig. 1. 1, *Bam*NI fragments of ρ 11C3a DNA; 2, untreated ρ 11C3a DNA; 3, fragments of ρ 11C3a DNA generated by purified *Bam*NI and purified *Bam*Nx; 4, remaining activity of *Bam*Nx after the preincubation with 25 μl of the crude extract from uninfected N cells; 5, inactivation of *Bam*Nx by the preincubation with 25 μl of the crude extract from ϕ NR2rH-infected N cells. 4 and 5 also show that *Bam*NI activity still remains in ϕ NR2rH-infected N cells. 6, *Bam*NI fragments of ρ 11C3a DNA.

in the extracts from ϕ NR2-infected and uninfected cells but not in the extract from ϕ NR2rH-infected cells (data not shown). We concluded, therefore, that ϕ NR2rH infection caused the inactivation of *Bam*Nx.

*Bam*Nx, purified according to the method described by Shibata and Ando¹¹, was preincubated for 15 min at 37°C with various amounts of the crude extract, and the remaining activity of *Bam*Nx was determined by Agarose gel electrophoresis. *Bam*Nx activity was seriously inhibited by the preincubation with crude extract prepared from ϕ NR2rH-infected N cells (Figs 2 and 3). This suggested that the *Bam*Nx inhibitor appears after the ϕ NR2rH infection, and that this *Bam*Nx inhibitor is responsible for overcoming the host-controlled restriction-modification system of strain N.

In order to reveal the molecular function of the *Bam*Nx inhibitor in detail, it will be necessary to purify it, but our preliminary data indicate that it is proteinous and inhibits *Bam*Nx specifically.

Phage ϕ NR2rH, like T3 and T7, has the function of overcoming the host-controlled restriction-modification system. The restriction endonucleases overcome by T3 and T7 belong to type I. Phage ϕ NR2rH may be the first example of a shape overcoming the type II restriction endonuclease of its host strain. A similar phenomenon might be found in further studies, and if so, it would have biological importance in host-parasite interactions.

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Dependence of protein synthesis on RNA synthesis during the early hours of germination of wheat embryos

WHEAT EMBRYO germination is characterised by an increase in fresh weight during the first 40 min of imbibition, followed by a lag period of 5 h and finally, a phase of cell elongation during which fresh weight increases at a sustained rate¹. An essential step in seed germination is *de novo* synthesis of proteins, but for the first 40 min it is mediated by preformed mRNA transcribed during embryogenesis². Previous reports have shown that although there is a 2.5–3-fold increase in the relative rate of protein synthesis during the lag period (40 min–5.5 h)³, there is no appreciable change in the rate of RNA synthesis^{4–7}. With the recently developed sensitive assays for ribonucleoside triphosphates^{8,9}, the rate of RNA synthesis and its mode of regulation in early germination of wheat embryos was re-examined¹⁰. In contrast to previous findings^{4–7}, our results clearly show a two- to threefold increase in the rate of RNA synthesis during the lag period of wheat embryo germination. This increase in RNA synthesis could conceivably account for the threefold increase in the rate of protein synthesis⁴ and raises the possibility that protein synthesis may depend on RNA

Fig. 1 Isolated wheat embryos (100 µg) were germinated for 3 h in the presence or absence of α -amanitin. Ribosomes were isolated as described by Spiegel *et al.*⁵. Four A_{260} units of ribosomes were separated on a 17–42% linear sucrose gradient with a 0.5 ml sucrose (1.8 M) pad in a Beckman SW 50.1 rotor by centrifuging at 49,000 r.p.m. for 40 min. *a*, control; *b*, treated with 2 µg ml⁻¹ of α -amanitin; *c*, treated with 10 µg ml⁻¹ of α -amanitin.

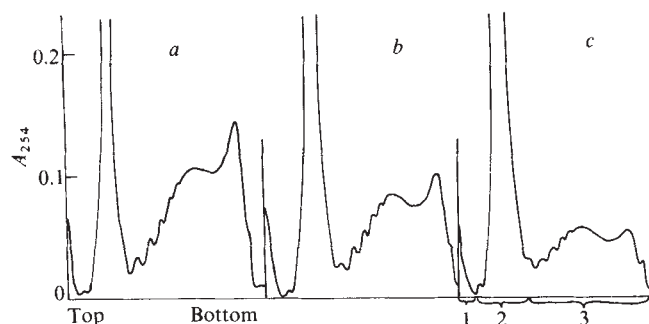


Table 1 Effect of α -amanitin on RNA(*a*) and protein (*b*) synthesis

<i>a</i>					
α -Amanitin added	[5- ³ H]uridine incorporation		Total mRNA		Ribosome-associated mRNA
(µg ml ⁻¹)	c.p.m.	%	c.p.m. per µg	%	c.p.m. per µg
0	27,600	100	13,600	100	19,500
2	17,300	63	10,600	78	17,800
4	14,100	51	—	—	—
10	9,000	33	7,300	54	12,700

<i>b</i>					
α -Amanitin added	[4,5- ³ H]leucine incorporation		Polysome content	Active ribosome	
(µg ml ⁻¹)	c.p.m.	%	%	c.p.m. per 0.1 A_{260} units	%
0	98,200	100	100	31,100	100
2	58,900	60	79	25,500	82
4	42,200	43	—	—	—
10	20,600	21	54	17,400	56

a, Isolated wheat embryos were germinated as described previously for 3 h in the presence or absence of α -amanitin¹⁰. After 3 h of germination, 10 µCi of [5-³H]uridine was used to label 50 mg of isolated wheat embryos for 30 min. The 5% trichloroacetic acid-insoluble material was prepared as described previously¹⁰. 50 mg of germinated wheat embryos were ground with 0.4 ml of high salt buffer (1 M KCl, 0.1 M Tris-HCl (pH 7.6) and 10 mM EDTA) with a mortar and pestle. 2 ml of 1% SDS buffer (5 mM EDTA, 10 mM Tris-HCl (pH 7.6) and 1% SDS) was then added to the extract, mixed well and extracted twice with 3 ml of water-saturated chloroform-phenol (1:1, v/v). The aqueous phase was adjusted to 0.2 M ammonium acetate and the RNA was precipitated by 2 volumes absolute ethyl alcohol (18 h, -20°C). The precipitate was washed 3 times with 2 M LiCl, twice with 70% aqueous ethyl alcohol, dried and dissolved in 2 ml of sterilised water. The total RNA (6.25 µg) was translated in a wheatgerm extract S30 system¹⁵. Ribosomes were obtained from 100 µg of isolated wheat embryos germinated for 3 h as described by Spiegel *et al.*⁵. The ribosomal pellet was suspended in 2 ml of 1% SDS buffer and then mixed with 1 ml of regular buffer (5 mM EDTA and 10 mM Tris-HCl, pH 7.6). 400 µl of high salt buffer were added to the ribosome suspension. The resulting precipitate was removed by centrifugation. 3 ml of water-saturated chloroform-phenol were used to extract the aqueous phase. The RNA was prepared as described before. The wheatgerm extract S30 was used to translate 5 µg of ribosome-associated mRNA. *b*, After 3 h of germination, the embryos were labelled with 20 µCi of L-[4,5-³H(N)]leucine for 30 min. The 5% trichloroacetic acid-homogenate was prepared as described previously¹⁰, except that it had been incubated at 90°C for 15 min. The polysome content was obtained from Fig. 1 by measuring the area of the polysome region from absorbance compared with the fraction plot following sucrose gradient centrifugation. Ribosomal fractions were prepared as described above. Excess wheatgerm extract S100 (5 µl) was used for the completion of nascent peptidyl chains present in 0.16 A_{260} units of ribosome in the presence of 8 µM pactamycin.

synthesis after the first 40 min of germination. We describe here experiments undertaken in wheat (*Triticum aestivum* L.) embryos to study the effect of inhibition of RNA synthesis on the rate of protein synthesis. Our data suggest that protein synthesis depends on RNA synthesis, and that the substrate level of mRNA is not a rate-limiting determinant for protein synthesis during the early hours of germination.

After 3 h of germination, concomitant decreases in RNA and protein synthesis were observed in isolated wheat embryos treated with α -amanitin (Table 1, column 3). Measurements of the fractionation of ribosomes isolated from α -amanitin-treated samples on a 17–42% sucrose gradient revealed a decrease in polysomes and an increase in monosomes (Fig. 1). With α -amanitin at 2 µg ml⁻¹ and 10 µg ml⁻¹, the polysome content was depressed by 21% and 46%, respectively (Table 1b, column 4). Active ribosomes were quantified by allowing the nascent peptidyl chains of active ribosomes to complete polypeptide

synthesis in the presence of the initiation inhibitor, pactamycin. The treatment of α -amanitin at $2 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$ caused a decrease in the amount of active ribosomes by 18% and 44%, respectively (Table 1b, column 5). The possibility of a direct effect by α -amanitin on protein synthesis was eliminated by an *in vitro* study with a cell-free wheatgerm translation system (data not shown).

α -Amanitin probably inhibits the synthesis of mRNA which in turn may affect either the substrate level of mRNA or the cellular content of an essential factor(s) required for protein synthesis. After 3 h of germination, the cellular level of total mRNA was 22% and 46% lower than the control sample when treated with α -amanitin at $2 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, respectively (Table 1a, column 5). The amount of mRNA actually associated with ribosome in α -amanitin-treated samples was decreased to a lesser extent. When $2 \mu\text{g ml}^{-1}$ of α -amanitin was used in the incubation, the amount of ribosome-associated mRNA decreased by only 9% (Table 1, column 7). Such a small decrease in the ribosome-associated mRNA cannot fully account for the 40% inhibition of protein synthesis (Table 1b, column 3) and the 20% depression of polysome content (Table 1b, column 4). Therefore, the substrate level of mRNA does not seem to be the rate-limiting determinant for protein synthesis.

No obvious changes in the polysome profile were observed following treatment with α -amanitin (Fig. 1). This observation would suggest that normal loading of ribosomes on the mRNA can proceed once the mRNA is 'activated' for protein synthesis. A reduced initiation efficiency in protein synthesis has been observed in other cell systems in resting compared with proliferating cultures^{11,12}. It is possible that the formation of the first peptidyl bond is required by the essential factor(s), as the first dipeptide synthesis is a unique step in protein synthesis¹³. It has also been reported that protein synthesis is not regulated by the supply of mRNA in oocytes of *Xenopus laevis*¹⁴.

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Table 1 H-2 compatibility required for immunological protection against HSV-1: adoptive transfer to AQR B mice

Donors (H-2 haplotype), no. and type of spleen cells transferred	Survival at 14 d	Survival at 60 d
AQR (qkddd)		
5×10^7 normal	1/9	1/9
5×10^7 immune	11/11	11/11
$1-1.5 \times 10^7$ immune	10/12	10/12
5×10^6 immune	0/4	-
B10.BR (kkkkkk)		
5×10^7 normal	1/9	1/9
5×10^7 immune	9/9	9/9
$1-1.5 \times 10^7$ immune	3/12	3/12
B10.T(6R) (qqqqqd)		
5×10^7 normal	0/8	-
5×10^7 immune	7/10	2/10
$1-1.5 \times 10^7$ immune	4/11	0/11
B10.BR + B10.T(6R)		
$1.5 \times 10^7 + 5 \times 10^6$ immune	5/11	5/11
C57BL/6 (bbbbbb)		
5×10^7 immune	2/10	2/10
$1-1.5 \times 10^7$ immune	0/8	-

of the K and D regions of the H-2 complex of the mouse; and because protection against lymphocytic choriomeningitis¹, ectromelia² and influenza³ requires compatibility at K or D in adoptive transfer experiments, it follows that the subset mediates protective immunity against these viruses. I-region compatibility requirements have not hitherto been observed in viral infections, but are necessary for protection against the intracellular parasite *Listeria monocytogenes*. This suggests that T helper cells (T_H) and/or the lymphocytes responsible for delayed hypersensitivity and lymphokine release may be involved⁴ (these lymphocytes can provisionally be included within the T_H subset). Adoptive transfer studies in mice have clearly established a major role for T lymphocytes in recovery from infection with herpes simplex virus (HSV-1)⁵⁻⁷. The T-lymphocyte subclass or classes responsible for this protection are not known. *In vitro* cytotoxic T cells (T_C)⁸, and their primed precursors⁹ can be obtained from HSV-1 infected mice. The present study indicates that immune protection against HSV-1 can be conferred by immune spleen cells possessing either I or K-D compatibility, and that long-lasting protection is conferred only by cells with I-region compatibility.

Donor strains of mice (Table 1) were immunised initially by intraperitoneal injection of 10^5 plaque-forming units (PFU) of Kruger strain HSV-1, boosted with 10^7 PFU after 1 week and 10^6 PFU after 2 weeks (virus was grown on cell strain BHK-13, and stored at -70°C at a concentration of 10^9 PFU ml^{-1}). After 10-14 d, pooled sera from each strain of donor mice consistently showed antibody in dilutions of 4×10^{-4} by a solid phase radioimmunoassay. At this time teased spleen cells were obtained aseptically, washed three times with a modified Eagle's medium and injected into recipients in the numbers indicated in Table 1. Recipients were 12-16-week-old AQR mice which had been adult thymectomised at 4-6 weeks of age, and at 8-10 weeks had been irradiated (750-800 R) and reconstituted with $1-2 \times 10^7$ AQR bone marrow cells following anti-Thy-1.2 and complement treatment ('B' mice).

Two days after spleen cell transfer, 2×10^6 PFU of HSV-1 were injected intraperitoneally; this was a quantity of virus found to be lethal for AQR B mice, which were themselves 100-fold more susceptible to the virus than normal mice of the same strain. Times of death were recorded and fell into two distinct groups. Mice dying less than 14 d after HSV-1 developed a progressive paralysis and findings typical of HSV

MHC matching shows that at least two T-cell subsets determine resistance to HSV

DISTINCT T-cell subsets recognise antigens in association with different major histocompatibility complex (MHC) products, and this provides a means of identifying the subsets which operate in protective immunity against pathogens. Thus, cytotoxic T cells (T_C) recognise antigen in association with products

encephalitis⁶. A second group of mice died 30–60 d after HSV-1. Those mice surviving beyond 60 d were considered to have a lasting immunity.

As Table 1 indicates 5×10^7 syngeneic immune cells (AQR) and 5×10^7 immune cells with I-region compatibility (B10.BR) conferred a long-lasting protection; $1\text{--}1.5 \times 10^7$ AQR immune spleen cells were also protective, but this number of B10.BR cells were protective in only 3/12 mice; 5×10^7 immune spleen cells with K-D compatibility, B10.T(6R), were protective initially in 7/10 mice but only 2/10 mice survived 60 d; $1\text{--}1.5 \times 10^7$ B10.T(6R) immune cells were initially protective in 4/11 instances, but long-lasting immunity was not achieved in any mouse. An attempt at enhancing protection by combining immune cells from two strains of mice, one with I-region compatibility (1.5×10^7 B10.BR) and the other K-D compatibility (5×10^6 B10.T(6R)) was successful in only 5/11 mice; this effect was long lasting, but in numbers not significantly different from that obtained with B10.BR cells alone. Spleen cells from non-immunised donors and from an immunised strain without H-2 compatibility (C57BL/6) failed to provide protection. By contrast, 5×10^7 of C57BL/6 immune spleen cells provided 100% protection in 26 acutely irradiated, HSV-infected B6 mice (350–450 R); spleen cells from non-immunised C57BL/6 mice failed to protect (18/21 deaths in less than 14 d).

These results demonstrate the need for H-2 compatibility in immune protection against HSV-1 in adoptive transfer studies in mice. Immune spleen cells from a mouse strain with known high resistance to herpes infection C57BL/6 (ref. 10), and demonstrated capacity to protect acutely irradiated HSV-1-infected syngeneic mice, fail to protect in non-H-2-compatible recipients. The protection conferred by H-2-compatible immune spleen cells is mediated by T cells, as we have confirmed previous work⁷ showing that treatment of the transferred cells with anti-Thy-1.2 and complement abolished their protective effect (data not shown).

T-cell recognition of both I and K-D coded self-markers seems to be important in immune protection against HSV-1 in mice. I-region compatibility confers a long-lasting protection, whereas K-D compatibility confers only a temporary protection in most animals. These findings are consistent with a role for both T_H lymphocytes and T_C lymphocytes in the immune response to this virus, and complete protection is provided by T_H lymphocytes alone in sufficient numbers. It might be argued that T_C lymphocytes may also have played a part in long-term protection, through differentiation of precursors in the thymus of the K-D-incompatible host; however, this seems unlikely because such precursors are not known to occur among spleen cells. This is therefore the first demonstration that T_H cells may have a greater protective capacity than T_C in a virus infection. It should be emphasised that this study differs from the previous short-term studies using recoverable virus as an end point, which have demonstrated a need for K-D compatibility only. T_H cells may prove important in recovery from these infections, as *in vitro* experiments suggest¹¹. The interpretation of our long-term effects are open to question because of the problem of reaction to the alloantigens, but the significant point is that long-term protection was in fact obtained. We think it unlikely that the long-term protection can be attributed simply to an allogeneic effect, not only because of its duration, but also because it was obtained with K-D-incompatible rather than I-incompatible cells.

T_H could be providing protection in several ways, including helping T_C precursors or B cells, the elaboration of lymphokines leading to the recruitment of other cells, and the direct activation of macrophages⁴. The importance of mature macrophages as effector cells in recovery from HSV-1 infections has been stressed previously^{7,12,13}. Help for B cells in the production of anti-viral antibody is perhaps the most straightforward possibility; the fact that anti-Thy-1 treatment abolishes protection does not argue against this possibility, for such treatment would inactivate the helper cells known to participate in the secondary antibody response to HSV and other viruses¹⁴. The failure to

achieve a protection comparable to that with syngeneic cells by a combination of two cell sources, one with I-region and the other with K-D-region compatibility suggests that compatibility may be required between T_H and T_C to achieve complete protection with low numbers of transferred immune cells.

Immune spleen cells recognising K-D-coded self-antigens only are also initially protective, strongly suggesting that T_C cells play a part in the immune response to herpes *in vivo* as well as *in vitro*⁹. The failure of such adoptively transferred mice to survive indefinitely may be due to eventual loss of specifically immune short-lived lymphocytes, although other mechanisms such as a graft-versus-host response could not be excluded in the present study.

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Evidence for a specific B27-associated cell surface marker on lymphocytes of patients with ankylosing spondylitis

THE strong association of HLA-B27 with ankylosing spondylitis (AS) as well as an increased frequency of B27 in other seronegative arthropathies is well documented^{1–4}. Nevertheless, the significance and the molecular basis of this remarkable association is still unknown. Ebringer *et al.*^{5–7} reported that *Klebsiella pneumoniae* was present in the faeces of patients with AS more frequently than in those of healthy controls, and that there seemed to be some cross-reactivity between HLA-B27-positive lymphocytes and *Klebsiella* antigen. It is possible, therefore, that an infectious agent, probably an enteric organism, is involved in the pathogenesis of AS. We have therefore decided to examine the *in vitro* proliferative responses of peripheral blood lymphocytes to *Klebsiella* and to several enteric organisms. To examine the possibility of cross-reactivity between B27 and *Klebsiella* antigen, we raised antisera in rabbits against various isolates of *Klebsiella* and tested the cytotoxicity of these antisera on lymphocytes of AS patients and of normal controls. The results presented here strongly suggest

Table 1 Comparison of means of log₁₀ transformed stimulation indices to various antigens of B27⁺AS⁺ patients compared with B27⁺AS⁻ and B27⁻AS⁻ normal controls

Antigen	Mean $\pm$ s.e.m. of log ₁₀ transformed stimulation indices			<i>t</i> values		
	B27 ⁺ AS ⁺ (a)	B27 ⁺ AS ⁻ (b)	B27 ⁻ AS ⁻ (c)	a v b	a v c	b v c
<i>Klebsiella</i> 411	0.22 ($\pm$ 0.02)	0.54 ($\pm$ 0.02)	0.64 ($\pm$ 0.02)	3.2*	4.8†	0.9
<i>Klebsiella</i> 427	0.12 ($\pm$ 0.02)	0.58 ($\pm$ 0.02)	0.56 ($\pm$ 0.02)	5†	5.5†	0.2
<i>Klebsiella</i> 433	0.13 ($\pm$ 0.02)	0.47 ($\pm$ 0.04)	0.58 ($\pm$ 0.02)	3.4*	5.1†	0.01
<i>Klebsiella</i> 462	0.05 ($\pm$ 0.01)	0.38 ($\pm$ 0.04)	0.40 ($\pm$ 0.03)	3.4*	4.8†	0.11
<i>Klebsiella</i> 468	0.08 ($\pm$ 0.01)	0.46 ($\pm$ 0.02)	0.37 ($\pm$ 0.02)	4.2†	4.1†	0.9
<i>Staphylococcus</i>	0.60 ($\pm$ 0.01)	0.75 ($\pm$ 0.17)	0.79 ($\pm$ 0.04)	1.0	1.7	0.5
<i>Streptococcus</i>	0.96 ($\pm$ 0.03)	0.87 ($\pm$ 0.12)	1.0 ($\pm$ 0.02)	0.6	1.2	0.4
<i>Shigella</i>	0.88 ($\pm$ 0.03)	0.89 ($\pm$ 0.07)	0.94 ($\pm$ 0.04)	0.2	0.7	0.5
<i>Yersinia</i>	0.69 ($\pm$ 0.02)	0.7 ($\pm$ 0.1)	0.72 ($\pm$ 0.01)	0.03	0.3	0.2
Phytohaemagglutinin	1.9 ($\pm$ 0.02)	1.8 ($\pm$ 0.03)	1.8 ($\pm$ 0.03)	1.23	1.1	1.0

The distribution of the stimulation indices for each antigen and each group of individuals was normalised by log₁₀ transformation and the means of the log₁₀ transformed values compared using the Student's *t* test (* *P* < 0.01, † *P* < 0.001). Peripheral blood lymphocytes were obtained from groups of B27-positive patients with ankylosing spondylitis (B27⁺AS⁺, *n* = 25), B27-negative normal controls (B27⁺AS⁻, *n* = 37) and B27-positive normal controls (B27⁻AS⁻, *n* = 13). The cells were separated from heparinised blood by centrifugation in Ficoll-Paque (Pharmacia) for 25 min at 580g. The leukocyte band was removed and washed with RPMI 1640 (Flow) medium containing 2 ml heparin (1,000 units ml⁻¹), 10 ml IM HEPES (Sigma) buffer, 5 ml L-glutamine (200 mM) and 2 ml penicillin-streptomycin (5,000 IU, 5,000 µg ml⁻¹; Flow) per 500 ml of RPMI medium. The leukocytes were suspended in RPMI medium containing 10% human AB serum and centrifuged for a further 10 min at 180g. The cells were then resuspended in RPMI medium containing 20% AB serum and adjusted to a cell concentration of 10⁶ mononuclear cells per ml of medium. Bacterial suspensions were adjusted to either 10⁶ (*Klebsiella*) or 10⁹ (*Staphylococcus*, *Streptococcus*, *Yersinia* and *Shigella*) organisms per ml RPMI containing 20% AB serum. Lymphocytes (5 × 10⁴ in 50 µl) were cultured in round-bottomed Linbro culture plates (Linbro) with 50 µl of the bacterial suspension (5 × 10⁴ to 5 × 10⁶ organisms) and 50 µl of RPMI containing 20% AB serum. All cultures were carried out in triplicate. In addition to the bacterial suspensions, the lymphocytes of each individual were cultured with phytohaemagglutinin (10 µg per well). The lymphocyte cultures were incubated at 37 °C for 6 d. On day 5, 1 µl of ³H-thymidine (1 µCi) was added to each well and the cells collected about 24 h later, with a Skatron (Lierbyen) multiple cell culture collector. The radioactivity of the cells was measured in a liquid scintillation counter (Packard, TRI-CARB, model 2002). Results are expressed as stimulation indices: c.p.m. with antigen per c.p.m. without antigen.

Table 2 Antiserum to *Klebsiella* sp. (427) lyses lymphocytes of B27⁺AS⁺ patients but not of B27⁺AS⁻ or B27⁻AS⁻ healthy controls

Serum	Absorption	Target lymphocytes			
		B27 ⁺ AS ⁺	B27 ⁺ AS ⁻	B27 ⁺ AS ⁻	B27 ⁻ AS ⁻
Anti-B27	0	44	0	67	0
Anti- <i>Klebsiella</i> 427	0	69	1	11	10
Anti- <i>Klebsiella</i> 427	B27 ⁺ AS ⁺	13	nt	2	7
Anti- <i>Klebsiella</i> 427	B27 ⁺ AS ⁻	73	nt	10	3
Anti- <i>Klebsiella</i> 427	B27 ⁻ AS ⁻	64	nt	13	2
Anti- <i>Klebsiella</i> 468	0	11	12	6	13
Anti- <i>Klebsiella</i> 462	0	2	6	8	0
Anti- <i>Klebsiella</i> 433	0	8	7	12	3
Anti- <i>S. typhimurium</i>	0	5	2	0	5
Anti- <i>S. typhi</i>	0	3	0	0	2
Anti- <i>Streptococcus</i> Group A	0	19	18	3	7
Anti- <i>Shig. dysenteriae</i>	0	2	0	4	5

Antisera to formalinised suspensions of various bacteria were tested on Ficoll-sedimented peripheral blood lymphocytes (see legend to Table 1) of B27⁺AS⁺ and B27⁺AS⁻ patients as well as B27⁺AS⁻ and B27⁻AS⁻ unaffected individuals in a ⁵¹Cr release assay. About 10 × 10⁶ lymphocytes were incubated in 1 ml of RPMI 1640 (Flow) containing 10% human AB serum and 100 µCi of ⁵¹Cr (specific activity: 200–500 µCi per µg, Amersham) for 60 min at 37 °C. The cells were then washed twice with RPMI + 10% AB serum and resuspended at a concentration of 3 × 10⁶ cells per ml. 100 µl of the ⁵¹Cr-labelled cell suspension were added to 10 × 75-mm plastic tubes and incubated with 100 µl of antiserum at 24 °C for 30 min. Rabbit complement (100 µl) was then added to each tube and the incubation continued for a further 60 min. The cells were centrifuged at 2,000 r.p.m. for 15 min and 100 µl of the supernatant was counted in a Packard auto-gamma scintillation spectrometer. The amount of radioactivity in the samples was compared with radioactivity present in an equal volume of the supernatant from tubes containing cells in medium plus complement only, and with that present in tubes containing cells which were frozen and thawed three times. The results are expressed as the percentage of maximum ⁵¹Cr released, which was calculated as follows:

$$^{51}\text{Cr release \%} = 100 \times \frac{\text{Radioactivity released by antiserum} - \text{radioactivity released in absence of antiserum}}{\text{Radioactivity released by frozen and thawed cells} - \text{radioactivity released in absence of antiserum}}$$

The results shown are of a typical experiment on the cells of single individuals for each category. The antibacterial activity on the rabbit sera was demonstrated by the slide agglutination technique. Both the absorbed and unabsorbed antibacterial antisera were tested at a dilution of 1:8; anti-B27 antiserum was used undiluted. Anti-*Klebsiella* 427 antiserum was absorbed twice by mixing 100 µl of antiserum with 30 × 10⁶ packed peripheral blood lymphocytes for 2 h at 24 °C. The absorbed serum was centrifuged at 10,000 r.p.m. for 30 min and diluted (1:8) with RPMI + 10% AB serum before testing.

that *Klebsiella* antigens cross-react with a gene product closely associated with HLA-B27 or with a *Klebsiella*-associated B27 antigen in AS patients.

As patients with AS have diminished T-cell responses to *Yersinia in vitro*⁸, we wondered whether there was a similar impairment of *in vitro* T-cell proliferation in response to other bacterial antigens. Lymphocytes were obtained from B27-positive patients with definite ankylosing spondylitis (by Rome and New York criteria⁹) as well as from B27-positive and B27-

negative healthy controls. Bacterial cultures were isolated from faecal samples (*Shigella flexner* and *Klebsiella*, isolate numbers 411, 427, 433, 462 and 468), a wound swab (*Staphylococcus* sp.) and a throat swab (*Streptococcus* sp.). *Yersinia enterocolitica* (NCTC no. 10598) was also used (American Type Culture Collection). Bacteria were killed by adding 0.25 ml of 40% formaldehyde to a 20-ml suspension of the organisms (10⁹–10¹⁰ per ml) in nutrient broth. The formalin-killed bacteria were washed twice with sterile 0.9% NaCl and stored at -20 °C.

Table 3 Lymphocytotoxic activity of Anti-*Klebsiella* 427 antiserum for lymphocytes from AS patients and from normal controls

Diagnosis	B27 positive	No. positive/no. tested
Definite AS	Yes	21/23
Probable AS	Yes	3/3
Definite AS	No	0/3
Normal	Yes	0/13
Normal	No	0/22

Diagnosis was based on Rome and New York criteria.

The data in Table 1 show that B27-positive patients with AS as well as B27-positive and B27-negative healthy controls respond equally well to phytohaemagglutinin *in vitro*. Similarly, the responses of individuals, in all three groups, to *Staphylococcus*, *Streptococcus*, *Y. enterocolitica* and *Shig. flexner* are not significantly different. Commercially obtained preparations of *Salmonella typhimurium* H specific and *S. typhi* H (Wellcome) failed to stimulate the lymphocytes of any of the individuals tested (data not shown). By contrast, B27-positive individuals affected with AS have a significantly lower *in vitro* responsiveness to *Klebsiella* (isolates 411, 427, 433, 462 and 468) as compared with B27-positive and B27-negative healthy controls. These observations, together with those of Ebringer *et al.*, suggest a possible cross-reactivity between *Klebsiella* antigens and HLA-B27, or the product of a closely linked gene. Alternatively, the lower lymphocyte responses to *Klebsiella* in AS patients may reflect an impaired processing of antigen by macrophages or the production of suppressor cells and/or suppressor factors by the lymphoid cells of affected individuals. These possibilities are now being studied.

In an attempt to gather further information on a possible cross-reactivity between *Klebsiella* and B27, we raised antibodies in rabbits to formalin-killed faecal isolates of *Klebsiella* (427, 433, 462 and 468) and to formalinised suspensions of *S. typhimurium* H specific and *S. typhi* H.

Rabbits (3–5 kg) were injected intravenously in the marginal ear vein with about 10^9 formalin-killed bacteria in normal saline (2 ml). Two weeks later, the animals received 10^9 – 10^{10} organisms in 2 ml of complete Freund's adjuvant (Difco) intramuscularly into multiple sites in the thigh and the nuchal skin. After 10–14 d the rabbits were boosted intravenously with 1 ml of bacterial suspension (10^8 – 10^9 organisms) in normal saline. Two weeks after the last injection, they were bled and the serum stored at -20°C . In some cases the rabbits were boosted intravenously at 2- to 3-weekly intervals (for periods up to 6 months) with about 10^8 organisms in 1 ml saline, and bled 1 week after each injection.

Table 2 shows the results of a typical experiment on the cells of single individuals for each category. The data indicate that an antiserum to *Klebsiella* 427 lyses the lymphocytes of B27-positive patients with AS but not those of B27-negative individuals with active disease. By contrast, the lymphocytes of B27-positive and B27-negative unaffected individuals are not lysed by an antiserum to *Klebsiella* 427. Furthermore, the cytotoxic activity of antiserum 427 may be removed only by lymphoid cells from B27-positive patients with AS but not by lymphocytes from B27-positive or B27-negative normal controls. Antisera to other isolates of *Klebsiella* (468, 462 and 433) produced virtually no lysis of any of the target lymphocytes. Similarly, antisera to *S. typhimurium*, *S. typhi*, *Streptococcus* Group A and *Shig. dysenteriae* (both Wellcome) gave <20% ^{51}Cr release from the four categories of lymphocytes. Preliminary results (data not shown) indicate that antisera to *Y. enterocolitica* and *Staphylococcus* also fail to lyse the lymphocytes of affected and normal individuals. It is of interest that only one isolate of *Klebsiella* (427) gave a persistently high percentage ^{51}Cr release from the cells of B27-positive affected patients, whereas sera raised against the other three isolates produced only about 10% lysis. This suggests either that only some strains of *Klebsiella* cross-react with the putative B27-associated gene

product in patients with AS or that *Klebsiella* 427 is more immunogenic in the rabbit than the other isolates tested. However, the latter possibility seems less likely, as rabbits boosted with isolates 468, 462 and 433 at 3-weekly intervals over a period of 4–6 months failed to produce detectable cytotoxic antibodies reactive with the lymphocytes of AS patients bearing the B27 allele.

Table 3 summarises the data on the reactivity of anti-*Klebsiella* 427 antiserum with lymphocytes from affected and normal individuals. The results strongly suggest cross-reactivity between some *Klebsiella* antigens and a gene product closely associated with HLA-B27 or possibly with genetically- or *Klebsiella*-modified B27 alloantigen complex in AS patients. The failure of anti-*Klebsiella* 427 antiserum to lyse cells from B27-negative AS patients suggests that B27 is an essential component of this hypothetical alloantigen-*Klebsiella* complex. Arnason *et al.*¹⁰ have provided evidence that the predisposing determinant in AS may be either a modified B27 or a gene located on the Bf (properdin factor B) side of the HLA-B locus. This evidence was based on the rationale that if the predisposing locus were on the same side of the HLA-B locus as the Bf locus, B27-Bf haplotypes in those affected should be more uniform than in those not affected. On the other hand, if the B27 alloantigen predisposed directly there would be no reason to expect any difference in the incidence of the Bf alleles in the affected minority and the non-affected majority who carry the B27 allele. These authors found that patients with AS were Bf⁺, whereas 23 of the controls (individuals with B27 but without symptoms) had Bf⁺ and 23 carried Bf⁻.

In the murine system, the concept of 'altered self' has been extensively investigated by Zinkernagel *et al.*¹¹, using as their model the adoptive transfer of T-cell immunity to the bacterium *Listeria monocytogenes*. These workers showed that immunity to the bacterium can be successfully transferred only if the donor and recipient share a portion of the I (immune) region of the mouse H-2 complex. The T cells involved in this genetically restricted transfer seem to be specific either for a complex of 'self' in association with *Listeria* antigen or for bacterial antigen which is expressed on the cell surface and which is modified by I region-linked enzymes^{12,13}. It remains to be determined whether a similar modification of the B27 allele, by an enteric organism, leads to the appearance of cytotoxic or suppressor T cells, and whether the activity of these cells is restricted by the human equivalent of the murine I region.

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reviews

Sussex school of perception

A. W. Still

Perceptions and Representations: The Theoretical Bases of Brain Research and Psychology. By K. Oatley. Pp. 262. (Methuen: London, 1978.) £7.

THIS is a non-technical exposition of what might be called (though probably not by its members) the Sussex school of perception. In 1959 Stuart Sutherland, following Lashley, set out the problems posed for any theory of perception, and many of his colleagues at Sussex, including the author of this book, contributed to the solutions subsequently offered. The problems arose because the invariants of perception are not reflected by invariants in the stimulus. Objects look the same from different aspects, for instance, and wherever their image falls on the retina, while the information in the stimulus may be drastically incomplete, but still recognisable, as in a cartoon figure. The Sussex solution is found in artificial intelligence, a branch of computer science which has created programmes to compute "picture descriptions". These programmes are based upon the spatial configurations of the parts of the picture. Physically different stimuli (or cues, the term preferred by Dr Oatley), such as pictures of the same scene from different aspects, can give rise to similar descriptions or representations, and can thereby receive a common classification (but they may not, depending upon the "purposes" incorporated in to the programme; thus there is an attractive freedom from control by the stimulus). Dr Oatley gives a very readable account of these views and his book will be of great value to students of psychology, as well as to anyone interested in the way computer science has influenced psychology.

He begins historically by tracing the background in reflex or stimulus-response theory. It is this theory which poses the problems recognised by Lashley, and which are unanswerable in its own terms. To develop more appropriate terms, Dr Oatley explores the idea of "emergent" properties, through an account of his own work on feedback mechanisms in hunger and thirst. Properties of a system "emerge" when they cannot be deduced from the "fundamental units" of the system.

Thus "properties of the feedback loop, control, regulation and entrainable oscillation clearly emerge as properties of organisation of processes in a feedback loop, not the individual processes themselves". Likewise, representational properties of mind emerge from physical elements, and artificial intelligence is an analogue of this process. By anchoring the concept to feedback loops and computer technology, "emergence" loses its mystical tinge, and mind can emerge respectably as matter organised in particular ways.

There are some clear accounts of artificial intelligence programmes, which are made accessible to the general reader without being watered down. These programmes can provide answers to the problems posed by stimulus-response theory, but do they provide an accurate model of visual perception? The evidence is sparse. True, one need only observe one's own thought processes to see that many perceptual tasks (such as Shepard and Metzner's experiments on puzzles the solutions of which involve imaging rotations of pictured solids) are solved by mental manipulations that can be incorporated into artificial intelligence programmes. But is all visual perception of this kind? The answer given by this book is "yes", perception involves the solution of a kind of puzzle, as retinal cues are incomplete, degenerate, often ambiguous, and sometimes downright misleading. But are they?

A questionable premise to the argument is that the eye is like a camera. This view is encouraged by, and encourages, experiments on perception of pictures (rather than objects) which have to be interpreted or classified. If the eye is a camera (albeit far from perfect) the picture is imaged on the retina, so the interpretation of the picture is, in effect, an interpretation of the retinal information. But what is the image and what is the retinal information, for a monkey leaping through trees or a skier weaving his way amongst trees? If the static image of a picture projected on to the stationary eye of a laboratory subject is degenerate, what can we say about the information on the retina of a moving animal? If the computations in the interpretation of a static image are complex, what are the computations

necessary for an image that is rapidly changing? Much more complex, obviously, and yet visually guided movement, often rapid and of great refinement, occurs throughout the vertebrates, and therefore amongst animals that show little ability to interpret pictures.

Perhaps artificial intelligence will find the answer to this; it is trying, and the difficulties are admitted, though not seriously considered by Dr Oatley. But there is a powerful alternative answer to the problem, also not seriously considered in this book. J. J. Gibson has shown how the invariants of perception might, after all, be reflected in the visual environment, especially if we take account of (rather than forget) the movements of the retina with respect to that environment. This involves giving up the eye-camera analogy and considering the environment in relation to an animal moving through it. The retinal projections will be transformed over time, and it is in these transformations that, if Gibson is right, important invariants of perception can be found. This is a truly new theory, whereas the picture description theory, far from being a modern reaction to stimulus-response theory as suggested by Dr Oatley, has its roots in the long philosophical history of perceptions being constructed out of sensations, sense-data, stimuli, or sensory inputs. The famous straight stick which looks bent in water is the direct ancestor of all the puzzle stimuli and ambiguous figures with which psychology textbooks abound. In both cases it is falsely concluded that because stimuli are sometimes misleading they must always be so, at least potentially.

These are possible criticisms of the Sussex school of perception, which, being a school, is naturally one-sided, and whether the criticisms are valid or not Dr Oatley's book remains an excellent statement of this one-sided theory. The shakiness of the theory's foundations are all the better exposed by the clarity of the book, especially as Dr Oatley himself expresses doubts in the last chapter. The doubts are general, about science as a paradigm for psychology. It has great value, but is limited, and does not, for instance, help in the conduct of personal relations. A suggested answer to these

doubts lies not in less but more reliance on computers, in the form of the "convivial computing" of Papert's LOGO project. The aim of the project is to use computers as teachers, whose pupils will remain free of the hangups and fears of appearing foolish that human teachers frequently inspire. It is an impressive project, but surely not an answer to Dr Oatley's doubts. Yet what answer could he give? Perhaps there is none within his framework of physiological psychology and artificial intel-

ligence. This may explain his choice of a quotation from Doris Lessing at the beginning of the book: "And when a book's pattern and the shape of its inner life is as plain to the reader as it is to the author—then perhaps it is time to throw the book aside, as having had its day, and start again on something new." □

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Protein turnover

Protein Turnover in Mammalian Tissues and in the Whole Body. By J. C. Waterlow, P. J. Garlick and D. J. Millward. Pp. 804. (North-Holland: Amsterdam, New York and Oxford, 1978.) £120; Dfl.276.

THIS is a scholarly book in the best sense of that term: Professor Waterlow and his colleagues know the subject matter thoroughly, think very clearly and write very well. It is an expensive volume, but the isotopically labelled materials you need to study protein metabolism in the whole animal are also expensive, and most workers in this difficult field could easily save the cost of the book by reading it carefully, and then designing their experiments more wisely.

A frontispiece to the book indicates a "scientific heritage" going back to the mid-eighteenth century, but the genealogy is not one of orderly succession. In the late 1930s Schoenheimer and his colleagues made an astonishing leap forward by showing that metabolic precursors of all kinds, and in particular amino acids, could be labelled with stable isotopes, and thus that their metabolism in whole animals and tissues could be followed. The technical problems which had to be overcome to carry out this work were so great that no other laboratory was able to carry on the torch, but as a result of Schoenheimer's work, biological scientists learned to regard the body less as a fixed machine which ran on dietary fuel, and more like a military regiment in which the structure is in dynamic equilibrium with the fuel—in this case, the recruits.

It has proved unexpectedly difficult to continue where Schoenheimer left off. In the past 20 years techniques for measuring both radioactive and stable isotopes have greatly improved. It is deceptively easy to carry out tracer studies with labelled amino acids, but all too often they are impossible to interpret. Good tracer studies need many skills, and if the investigator's grasp of biochemistry or mathematics is inadequate, mere technical accuracy is of no use. This book concentrates on the biochemistry and mathe-

The first section (164 pages) is on processes: a review of protein synthesis, degradation, and amino acid pools and transport. The key sentence is: "No measurement of the rate of incorporation of a labelled amino acid into protein can be used to calculate the rate of protein synthesis unless the specific activity of the free amino acid at the site of protein synthesis is also known." The second section, on methods (263 pages) starts with a useful introduction to mathematical models. Non-mathematicians should read this, as the study of anything so complex as whole body protein turnover requires models, and the more you know about

their advantages and limitations the more likely you are to use them appropriately. The next chapters deal with methods for measuring protein turnover in the whole body by observing free amino acid labelling, in individual tissues by observing incorporation of label into tissue protein, and some special cases such as isolated perfused organs.

The remaining 323 pages of text on results draws heavily on the work done by Professor Waterlow and his colleagues, and this concludes with a most stimulating chapter on directions of future research. There are great technical and intellectual challenges for the next generation of research workers, in a field ready for a new explosion of knowledge. The problem is that the body is much less homogeneous than a military regiment, or even an army, as there are many more types of protein, with different kinetics, than there are types of soldier. For those who take up the challenge to discover how and why protein is turned over in tissues and animals (including man) this book is an excellent guide.

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Seed development

Physiology and Biochemistry of Seeds in Relation to Germination. Vol. 1: Development, Germination, and Growth. By J. D. Bewley and M. Black. Pp. 306. (Springer: Heidelberg and Berlin, 1978.) DM90.

THE authors of this first volume of a two-volume work have concerned themselves with the physiology and biochemistry of seed development, germination and seedling growth. Approximately one-third of the book deals with seed structure and the development of the seed on the parent plant, and the remainder considers germination and seedling growth with particular attention to the mobilisation of reserves and the control of mobilisation. The interest in these topics and the use of seed and seedling material for much research in the plant sciences gives the subject of this volume a considerable importance for both teaching and research. Part of the work provides an excellent text for advanced students, one of its strongest points being its critical treatment of those dynamic aspects of seed development and germination, such as nucleic acid and protein synthesis and cereal aleurone layer studies, which have seen research work of a high quality in recent years. In areas in which there has been less distinguished recent work, such as the study of seed and seedling respiration and its control, the authors seem to

have been rather less critical and somewhat over-generous in their assessment of the literature.

The authors' aim for comprehensiveness has resulted in the book having some rather dull sections, for example, on structure and on the chemistry and biochemistry of reserve materials, so that parts of the book have the character of a reference work, a feature of which is in keeping with the rather high price of the volume. Of course such a slim volume cannot really provide a comprehensive coverage of the subject and therefore each chapter is followed by some more general references so that the reader can fill out the topic. One vital and rather difficult topic has received very inadequate attention: the question of the measurement of germination, which receives less than two pages and exhortations to read the literature. Something more authoritative was called for here.

There are few errors in this attractively produced book. Most of the Tables and Figures have originated fairly directly from the literature and I found most value in those that had been specifically produced by the authors. In volume two I hope that the authors will give more scope to their illustrative talents and I look forward to the early appearance of this volume and to its treatment of the real problem, that of seed dormancy.

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Amino acid transport

A Review of Amino Acid Transport Processes in Animal Cells and Tissues. By J. Lerner. Pp. 234. (University of Maine at Orono Press: Orono, Maine, 1978.)

AMINO ACIDS are chemically stable and easy to assay. With rapidly expanding techniques of microperfusion, micro-puncture and cell fractionation one might have been forgiven for thinking that most of what was worth knowing about these substances would soon be discovered. This has clearly turned out not to be the case for, despite a torrent of data produced in the past two decades, relatively little is known about the fundamental aspect of transport into and out of cells. The literature is massive, with up to 100 original articles and some half-dozen reviews appearing annually. Professor Lerner is to be congratulated therefore on tackling the mammoth task of reviewing this field and providing a valuable synopsis of current information and concepts.

He accomplishes his task in 177 pages of readable text which cites just over 900 references up to 1976. The index is

excellent and the 15 chapters (some of which are a single page in length) are well laid out. Most data described have been derived from marine invertebrates, insects, parasites and mammals (with detailed considerations of transport in the gut, central nervous system and to a lesser extent the kidney), with the intact organism and isolated preparations considered in detail. There is adequate reference to other tissues and isolated cultured cells, with the differing specificity of particular transport systems according to organ and species suitably highlighted.

Perhaps more than anything else, the complex interactions of the amino acids themselves on their own transport and the paucity of synthetic non-competitive specific inhibitors, have hindered the elucidation of mechanistic details. However, of the effects known, those of hormones, mercury, nucleotides, amino acid analogues, vitamins and sugars are suitably described. The important influence of sodium ion concentration is tackled in detail, although rather surprisingly without reference to the elegant work by Ullrich and his collaborators on the isolated rat renal tubule.

Within the context of the whole organism there are interesting observations on the adaptation and regulation of transport and how transport changes

during foetal development. Contributions from the clinical front include the inborn errors of metabolism, dietary deficiencies, malabsorption and starvation.

This volume is comprehensive but strictly a review. The theme is usually clear, but on occasions the book would benefit from an overall synthesis and some comment. Sometimes the argument becomes diffuse, points of interest or inconsistency are not highlighted, and there is little speculative comment. It is disappointing (but also indicative of our knowledge) that proposed mechanisms of transport only occupy 10 pages and there is no mention of any possible role for transmembrane peptides which are known to be important in other transport systems. However, despite these observations the work will undoubtedly be of considerable use to research workers in and students of biochemistry, neurochemistry, comparative physiology, nutrition, regulation biology and I think especially clinical science. It should find a place in all laboratories working on amino acid metabolism.

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Upper atmosphere, ionosphere and magnetosphere

Handbook of Astronomy, Astrophysics and Geophysics. Vol. 1: The Earth. 1. The Upper Atmosphere, Ionosphere and Magnetosphere. Edited by C. W. Gordon, V. Canuto and W. I. Axford. Pp. 412. (Gordon and Breach: London, 1978.) £35.

As this particular volume represents the first of forthcoming volumes on a very wide range of astronomy, astrophysics and geophysics, a few general comments may be in order on the general editorial matter. First, it is not clear whether or not this particular volume (volume 1) is supposed to cover the entire subjects of "The Upper Atmosphere, Ionosphere and Magnetosphere." If other volumes are planned for the above subjects, it should have been mentioned in an editorial note. How many volumes are planned? Is general subject matter for each volume decided? Or is this going to be a collection of review papers as they come along?

Secondly, the volume consists of six articles, a mixture of comprehensive articles covering a very wide range of topics and those covering rather specific topics. Thus, the general editorial policy for the general planning of this handbook is not very clear. Further, there does not

seem to be a uniform format: some have an abstract, some do not. Some articles have one page for the title and some do not. For an extensive article, it would be helpful to have at least a Table of Contents on the cover page. Also, if it is going to be truly a *Handbook* it should have at least a subject index.

Among the articles in the first volume "Hydrogen in the Upper Atmosphere" by B. A. Tinsley, "Auroral Particle Precipitation . . . Observations" by B. A. Whalen and I. B. McDiarmid, and "The Physical Mechanisms of the Inner Van Allen Belt" by M. Walt and T. A. Farley are perhaps the most comprehensive and up-to-date ones in each subject area. The coverage of the article "The Equatorial Electrojet" by D. M. Cunnold is rather limited, and there is little mention of some of the recent developments, such as the relationship between the F region of the ionosphere and the equatorial electrojet. The article "Polar-Cap Absorption Observations and Theory" by G. C. Reid does not cover observations of solar protons in interplanetary space and in the magnetosphere. The coverage of J. R. McAfee's article "Electron Plasma Resonances in the Topside Ionosphere" is reasonable for this limited subject.

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Acetylcholine synthesis

Acetylcholine Synthesis in Neurons. By S. Tuček. Pp. 259. (Chapman and Hall: London, 1978.) £15.

In recent years several excellent books on cholinergic mechanisms have been published (for example, *Biology of Cholinergic Function*, edited by A. M. Goldberg, I. Hanin, Raven: New York, 1976; *Neuromuscular Junction Handbook of Experimental Pharmacology*, 42, edited by E. Zaimis, Springer: Berlin, 1976). As these books have covered many topics of cholinergic transmission in more or less detail, one might wonder whether there is a need for another book on acetylcholine. However, the present monograph aims to be an addition to, rather than a substitute for its forerunners, by giving a detailed analysis of many aspects of transmitter synthesis, a subject which has not yet received the degree of attention it probably deserves. In my opinion the book fulfils its aim in an elegant manner. In the first place it is extremely readable, if only it were for the fact that it measures less than 200 pages of text, in contrast to its more corpulent counterparts, where the busy reader may hesitate reading the full contents at leisure. If one takes into account its compact size the book is admirable in offering so much detail with such clarity. The author never seems to be in a hurry when explaining difficult matters; when there is no way out of a problem, as is often the case with conflicting evidence, he treats the subject with justified economy.

The book sets out with two chapters which nicely summarise what is known about choline acetyltransferase. Among other things the physical and enzymic properties of this protein are discussed, together with its presence in nerves and also in some non-nervous tissues, and its transport in cholinergic axons. Subsequently, there are two very informative chapters devoted to the supply of acetyl and choline moieties which finally form the acetylcholine molecule. The supply of choline and choline-containing substances to the nerves (with special attention to the brain) and the subsequent neuronal uptake of free choline are described, and the problem is discussed how, or rather in what chemical form, activated acetyl groups migrate through the mitochondrial membrane into the cell sap of the nerve terminal. These chapters are followed by a survey of what is known about the organisation and control of acetylcholine synthesis. This actually forms the body of the book, and is also the most interesting part.

In addition to synthesis, the storage and release of acetylcholine are briefly discussed, as there are good reasons to believe that storage and release are intimately connected with the synthetic process. According to the author the synthesis of acetylcholine is probably controlled by mass action, enough choline acetyltransferase being present to provide a rapid recovery from disturbances of equilibrium caused by release of transmitter. High affinity uptake of choline and release of activated acetyl groups by the mitochondrion may represent additional factors in the mechanism controlling acetylcholine synthesis. The turnover of acetylcholine in the living brain receives little attention in this chapter and perhaps some readers will regret this. A full discussion of this subject would have been redundant, because it has been extensively reviewed recently by I. Hanin and E. Costa in a chapter of *Biology of Cholinergic Function*,

cited above. The last chapter of the book treats, among other things, the changes of choline acetyltransferase during development and ageing, and during Wallerian degeneration.

The book provides an extensive list of references (papers as recently as 1977 are referenced), which, so far as I have been able to judge, covers practically all that has been published in the field described in the book. It is the kind of reference list which makes the bulk of one's private file of references suitable for the dustbin.

Overall the book is a most valuable source of information on a topic which has not recently been reviewed elsewhere. Further it has the merit of being of educational value for investigators entering into the cholinergic field.

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Freshwater fish production

Ecology of Freshwater Fish Production. Edited by S. D. Gerking. Pp. 520. (Blackwell Scientific: Oxford, 1978.) £23.50.

In 1966 a symposium on *The Biological Basis of Freshwater Fish Production* was held at Reading University, England, and the proceedings were published by Blackwells in 1967 as a volume carrying the same title. The book was in great demand and has sadly been out of print for some time. However, the overwhelming response to that volume stimulated the production of the present book. The title has been changed and, although it is still recognisable as a 'reprint' of the earlier edition with many of the same authors simply revising and up-dating their previous articles, it does have some new chapters, some new authors and a somewhat different approach. In addition some of the authors appearing in the first edition are absent from this one. Dr Shelby Gerking has been the editor of both volumes and has done a remarkably fine job. The present *Ecology of Freshwater Fish* is a much better work than its predecessor and, as the former was an excellent text, one can appreciate the high opinion the reviewer has of this present edition.

The book is divided into four main areas: vital statistics of fish populations; the fish population and its food supply; competition and social behaviour influencing production; and the contribution of fish production to human nutrition and well-being. The last section is most

welcome, and one worthy of the lengthy treatment it receives this time. It includes contributions on fish yield assessment of large lakes and reservoirs, the growing science of aquaculture, ecological aspects of warmwater fishpond management, and the contribution of freshwater fish to human food, the latter containing a most valuable collection of statistics and up-to-date information.

In the scheme of fish production, information on mortality is required, and one serious omission in this otherwise very complete book is any consideration of mortality from disease, parasitism and predation. Certainly the role of predaceous fish in ecosystems is dealt with in some length, but one would have thought that two papers, one on disease and parasitism, and one on predation by other animals, would have been a 'must'. There is considerable published work on the effects of disease and predation on both production and yield. Even in the first edition a paper on predation on fish by other animals was included, but for some reason this has been omitted this time.

The book is up to Blackwells usual high quality of production, and only one figure (Fig. 127, p320) is sub-standard. It should be in the library of every university department of biological sciences and on the shelves of freshwater fishery staff. It would also be a useful investment for undergraduate as well as postgraduate fisheries students.

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